APPLIED CHEMISTRY

A PRACTICAL HANDBOOK FOR STUDENTS OF HOUSEHOLD SCIENCE AND PUBLIC HEALTH

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VOLUME II.

FOODS

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PREFACE

THE reasons for the production of this book have been dealt with in the preface to Vol. I. Since the first volume was published the degree of B.Sc. (Household and Social Science) has been substituted for the diploma originally granted by the University of London, and the complete book is intended primarily for students taking the course in Applied Chemistry, which is one of the subjects for this degree.

In the time available for instruction in this subject it is obviously impossible to deal with a number of important branches of Applied Chemistry. It is not even possible to include the whole of the matter dealt with in these two volumes in the course of any one session. The present volume deals with certain branches of the chemistry of food and with the interpretation of the analytical results obtained. The subject of food is, of course, also dealt with in this College in connection with the instruction in Physiology, Hygiene, Bacteriology, and Household Work.

As in the case of Vol. I. a certain amount of theoretical matter is introduced, which will, we hope, enhance its value as a laboratory manual.

Some of the experiments described in the Chapter on the Cooking of Foods involve the use of cooking stoves, saucepans, etc. Such work cannot be conveniently carried out in the Chemical Laboratory, and in this Department special equipment for this purpose is provided in the Kitchen Laboratory (see Preface to Vol. I.). As in the previous volume, this section of the

work; being of a more specialised nature, is denoted by two asterisks.

We wish to express our thanks to Mrs. D. Jackman, B.Sc., for preparing some of the diagrams and for assistance in reading the proofs.

For the use of blocks for illustrations we are indebted to Messrs. A. Gallenkamp & Co. Ltd., Messrs. Baird & Tatlock (London) Ltd., and Messrs. F. E. Becker & Co.

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Chapter I.

COWS' MILK.

GENERAL CHARACTERISTICS.

This yellowish white opaque fluid, the specific gravityof which varies from about 1·027 to 1·035, contains substances in true solution, colloidal solution, and in suspension. It consists of a mixture of water, fat, carbohydrate, protein, and mineral matter; the total solids varying, as a rule, from 12 to 13 per cent. by weight of the milk. It should be noted that this percentage of solid matter is greater than that in certain solids used as vegetables, e.g. turnips. The colour of milk, which is due to the suspended fat globules, varies according to the breed of cow from which the milk is obtained, and upon the nature of the material on which the animal has been fed. The question of added colouring matter is dealt with on page 20. The fat globules, which have an average diameter of about 0·005 mm., are readily seen by means of a microscope. The amount of fat in different samples of milk varies from 2·5 to 7 per cent. by weight of the milk, but in unadulterated milk is very rarely less than 3 per cent., which is the legal minimum in this country.

The carbohydrate present in milk is lactose or milk sugar, $C_{12}H_{22}O_{11}$, and this substance is present in true solution. Owing to the ease with which lactose is converted into lactic acid, $CH_3 . CH(OH) . COOH$, milk, which may be either acid or alkaline in reaction towards litmus when first drawn from the cow, soon acquires a permanent acid reaction. The amount of lactose in cows' milk varies from 3 to 5 per cent. by weight of the milk.

The chief protein in milk is casein, which in combination with calcium and phosphate is present in colloidal solution to the extent of about 3 per cent. by weight of the milk. Lactalbumin, a soluble protein, is present to the extent of about 0·6 per cent., and other nitrogenous organic substances

are present in small amounts. The curdling which takes place when milk turns sour is due to the precipitation of the casein owing to the accumulation of lactic acid in the milk. The fat is also carried down mechanically when the casein is precipitated.

LEGAL STANDARD FOR MILK.

The legal standard in this country to which milk must conform is that it shall contain not less than 3 per cent. by weight of milk fat, and not less than 8·5 per cent. by weight of solids other than fat. In addition, milk must be free from preservative and added colouring matter. In the following pages methods are given for a complete examination of milk, although the determinations involved in finding out whether or not a given sample of milk conforms to the legal standards, as regards total solids and fat, are those of chief importance in connection with milk analysis.

DETERMINATION OF THE SPECIFIC GRAVITY OF MILK.

This determination should be made either by means of a specific gravity bottle or Westphal specific gravity balance. (See "Chemistry of Petroleum," * p. 110.) The specific gravity or relative density of milk at 60° F. (15·5° C.) will be found to be between 1·027 and 1·035 (water at 60° F. = 1).† It is, however, more usual to take the value for water as 1000, so that the specific gravity of milk is between 1027 and 1035, usually about 1032.

It should be noted that since milk is specifically heavier than water, addition of water to milk causes a *diminution* in the specific gravity. On the other hand, milk fat is specifically lighter than water, so that removal of cream from milk *increases* the specific gravity of the milk. The cream contains practically all the milk fat, together with water and other constituents of milk. Machine-skimmed milk, for example, has on the average a specific gravity of 1037. By proper admixture, therefore, of skimmed milk with water, a milk of specific gravity 1032, corresponding to that of genuine milk, may be obtained.

It will thus be obvious that no reliable evidence as to

* See list of reference books, p. 268.

† If the temperature of the milk is not exactly 60° F. a correction of the observed specific gravity must be made in order to obtain the value at 60° F. (See "Dairy Chemistry," by Richmond, for table of corrections.)

genuineness of a sample of milk can be obtained merely by a determination of its specific gravity, and information obtained by the use of a *lactometer* (a form of specific gravity hydrometer *) is quite unreliable. Thus in one form of lactometer one point (32) on the scale, which is graduated from 0 to 40, is marked M (whole milk), another (40) is marked S (skimmed milk), and the zero of the scale is marked W (water). The points are supposed to show the portion of the scale which will be in contact with the surface of the liquid when the lactometer is floating in whole milk, skimmed milk, and water respectively. It will be seen, however, from what has been stated above, that in a certain mixture of skimmed milk and water the point marked M, indicating whole milk, will be in contact with the surface of the mixture.

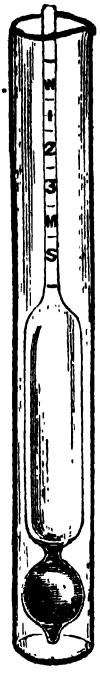


FIG. 1.—Lactometer.

Example.—

What volumes of skimmed milk, of specific gravity 1037, and of water must be mixed to give a milk of specific gravity 1032?

In 100 volumes of the mixture let x = volume of water.

Then $100 - x$ = volume of skimmed milk.

If 1 volume of water = 1000 parts by weight,

(i) x ,, ,, = $1000x$,, ,,

1 volume of skimmed milk = 1037 parts by weight,

(ii) $(100 - x)$,, ,, = $1037\,(100 - x)$.

Adding (i) and (ii)—

$x + 100 - x$ volumes of the mixture

= $1000x + 103700 - 1037x$ parts by weight,

i.e. 100 volumes of the mixture = $103700 - 37x$. ,, ,,

$\therefore$ 1 ,, ,, ,, = $\frac{103700 - 37x}{100}$,, ,,

But 1 volume of mixture is to equal 1032 parts by weight,

$$\therefore \frac{103700 - 37x}{100} = 1032,$$

* See "Chemistry of Petroleum," p. 114.

or $$37x = 103700 - 103200$$
$$= 500.$$
$$\therefore x = \frac{500}{37} = 13{\cdot}5.$$

Thus to make the required mixture of specific gravity 1032, in every 100 volumes 13·5 volumes should be water and 86·5 volumes of skimmed milk of specific gravity 1037.

DETERMINATION OF TOTAL SOLIDS.

Mix thoroughly the sample of milk. Weigh a flat-bottomed porcelain, silica, or platinum * basin with a short glass rod rounded at both ends, and introduce 5 c.c. of milk. Re-weigh the dish and milk. Heat the dish on a water bath, stirring frequently, until the milk residue appears free from water. Heat the dish in a water oven for an hour, cool in a desiccator, and weigh. Re-heat in the water oven for half an hour and re-weigh. Repeat the process until the weight is constant. Calculate the percentage by weight of total solids in the milk. For genuine milk the result will usually be from 12 to 13 per cent.

DETERMINATION OF ASH.

The residue obtained in the determination of the total solids is carefully incinerated over a Bunsen burner in a draught cupboard until a white ash remains. Any solid adhering to the glass rod is carefully scraped off during the ignition by means of a metal spatula. If the ash is heated very strongly sodium chloride may be lost by volatilisation. Cool the dish in a desiccator and weigh. Calculate the amount of ash which would be obtained from 100 gms. of milk. This will probably be about 0·75 per cent.

The ash should be tested for the presence of calcium and phosphate (see page 135).

DETERMINATION OF THE FAT IN MILK.

THE GERBER (CENTRIFUGE) METHOD.

In this process a measured volume of milk is treated in a tube (Fig. 2) with a measured amount of concentrated sulphuric acid of specific gravity 1·820 to 1·825. The acid first causes a precipitation of casein, which subsequently redis-

* If the ash in milk is to be determined after the total solids, the use of a platinum or silica basin is to be preferred.

solves. A measured amount of amyl alcohol (B.P. 124° to 130°.C., and specific gravity 0·815 to 0·818) is also added to the mixture to facilitate the separation of the fat, which is obtained as a layer on the surface of the liquid after the mixture has been whirled in a centrifuge. The upper part of the tube is so graduated that the percentage of fat in the milk is read off directly.

Place 10 c.c. of sulphuric acid (specific gravity 1·820 to 1·825) in each of two Gerber tubes. The pipette used for this purpose has two bulbs above the graduation mark to diminish the possibility of sulphuric acid being drawn into the mouth. Add carefully 11 c.c. of the well-mixed sample of milk (measured by means of an 11 c.c. pipette). The milk and acid should not be allowed to mix. Now add 1 c.c. of amyl alcohol to each tube.

Close the tube with a rubber bung, cover it with a duster, and invert several times until all the curd has dissolved.

Immerse the tubes in water at 70° C. for about ten minutes. If the surface of the liquid in the tube is not on the scale the rubber bung should be screwed in further.* Then centrifuge for five minutes. The tubes, two or four, must be placed in the brass tubes of the centrifuge with their stoppers towards the rim of the apparatus and opposite one another, or the centrifuge will not run properly and will be damaged.

The lid of the centrifuge must be replaced before the whirling is commenced. In cold weather it is advisable to place a *small* Bunsen flame under the tray of the centrifuge, but only *whilst this is in motion.*

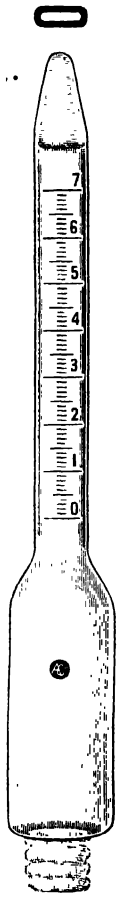


FIG. 2.—Gerber Tube, with cross-section of graduated part of tube above.

If, on removing the tube from the centrifuge, the *lower* level of the layer of fat is not on the graduated scale of the tube the rubber bung should be screwed in still further. Read off the percentage of fat from the scale, taking the *lowest* point of the meniscus as the upper reading. If there is a layer of

* If a badly made tube is being used it may be necessary to add a little more sulphuric acid to the cooled tube.

undissolved matter below the fat insufficient heating in the water bath is probably indicated.

The results obtained by this method are not as reliable as those obtained by any of the methods described below.

THE GOTTLIEB-ROSE METHOD.

In this process a known weight or measured volume of milk is treated with alcohol and ammonia, whereby the casein is first precipitated and then passes into solution. The fat may then be extracted by means of a mixture of ethyl ether (methylated ether *) and light petroleum (petroleum ether) and, after evaporation of the solvent, weighed. The object of using petroleum ether is to diminish the solubility of the lactose in the aqueous ethyl ether.

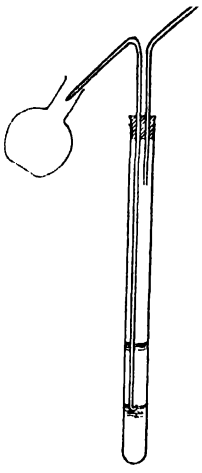


FIG. 3.

Weigh accurately a small beaker containing 5·5 c.c. of milk and a short glass rod. Pour the milk down the rod into a glass tube about 15 inches long and of $\frac{3}{4}$ inch diameter (Fig. 3). Re-weigh the beaker and rod. The difference in weight is the weight of milk taken for the experiment.

It should be noted that a pipette which is graduated to deliver a definite volume of water will not deliver an equal volume of milk, so that it is not quite accurate to take "5 c.c." of milk and calculate its weight from its specific gravity, although this method is adopted in some of the determinations which follow.

By means of a dropping pipette add 0·5 c.c. of a solution of ammonia, made up by diluting 0·88 ammonia solution with an equal volume of water. Mix the ammonia solution with the milk, add 5 c.c. of alcohol (95 per cent. by volume), and again mix the contents of the tube. Add 12·5 c.c. of ethyl ether, stopper the tube with a rubber bung, and mix the contents by inverting the tube three times. If the mixture is shaken too vigorously an emulsion may be formed which separates very slowly. Add 12·5 c.c. of petroleum ether (light petroleum) and again invert the tube three times.

* Owing to the highly inflammable nature of these substances the greatest care must be exercised in their use.

Support the tube vertically, and when the upper layer is clear, insert the rubber bung carrying the wash bottle tubes, as shown in the diagram. Transfer the ether-petroleum ether layer to a dry, weighed flask by blowing through the short tube. Repeat the extraction with three successive quantities of 20 c.c. of a mixture of ether and petroleum ether.

Distil off the ether and petroleum ether from the combined extracts in the weighed flask. The receiver should consist of a filter flask or distilling flask attached to the condenser by means of a cork. To the side tube of the receiver is attached a rubber tube, the end of which is below the level of the bench, to allow the removal of uncondensed ether vapour and prevent its accumulation near the flame (see Vol. I., p. 170). The distillate consisting of a mixture of ethyl ether and petroleum ether should be put into a bottle labelled "ether-petroleum ether residues." When all the ether has been distilled off, dry the flask in the water oven * for one hour, cool in a desiccator, and weigh. From the increase in the weight of the flask calculate the percentage by weight of fat in the milk.

To make sure that the flask contains only fat, wash it out several times with small quantities of petroleum ether, dry in the steam oven, and re-weigh.

THE WERNER-SCHMIDT METHOD.

In this method 10 gms. of milk are heated in a tube, similar to that used in the previous process, with 10 c.c. of concentrated hydrochloric acid until a dark brown liquid is obtained. If a boiling tube is used it may be heated over a flame, but if a thick walled tube be used it should be heated in a water bath. When the contents of the tube are cold 25 c.c. of ethyl ether are added and the tube, fitted with a rubber bung, is inverted three times to mix the contents. The ethereal solution of the fat is transferred by means of the wash bottle tubes into a *dry*, *weighed* flask.

The extraction is repeated with three successive quantities of 20 c.c. of ether. The combined extracts are distilled, as in the previous determination, and the flask dried in the steam oven, cooled in a desiccator, and weighed. The fat is then removed from the flask by repeated washing with small

* Although practically all the ether vapour should have been removed from the fat in the flask, it is advisable to turn off the gas burner under a hot-water oven, containing such flasks, before the door of the oven is opened; otherwise the accumulated ether vapour may become ignited.

quantities of petroleum ether. The flask is dried in the steam oven, cooled, and weighed. If the flask contained nothing but fat the weight now should be the same as the weight of the flask at the beginning of the experiment.

The weight of the flask *plus* the fat, *less* the weight of the flask, gives the weight of fat in the milk taken, from which the percentage of fat is calculated.

THE ADAMS (SOXHLET EXTRACTION) METHOD.

This method, which is applicable for the determination of fat in most substances, consists in extraction with ether by means of a Soxhlet apparatus, the construction of which is shown in the diagram (Fig. 4).

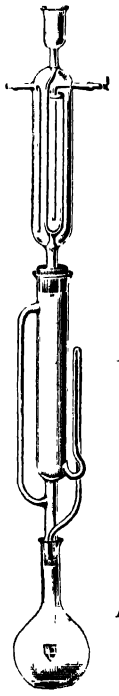


FIG. 4.—Soxhlet Extraction Apparatus.

Note.—For fitting up this apparatus corks and not rubber bungs must be employed.

A strip of filter paper about 2 ins. wide and 16 ins. long is rolled into a coil so as to fit loosely in the extraction tube A. Before rolling up the paper, however, a piece of thin wire or string is laid along the centre of it, so as to separate the layers of the roll. The end of the wire or string is tied round the roll and a loop is left so that the roll may be suspended in a beaker by means of a glass rod placed across the top. Place the roll of filter paper in the extraction tube and pour ether through the double surface condenser C until it siphons over from the extraction tube into the flask B. Pour more ether through the condenser until the extraction tube is again half full. Heat the flask B on a water bath or electric hot-plate, and regulate the heating so that the ether drops from the condenser into the extraction tube at the rate of about one drop per second.* Allow this operation to proceed until the ether has siphoned over about twelve times. The time required for the completion of this part of the process may be calculated by noting the time between two siphonings.

This preliminary treatment is to remove any fat contained

* As the extraction proceeds more and more fat accumulates in *B*, but this fat is of course non-volatile at the temperature employed.

in the filter paper and string. Remove the roll from the extraction tube after the twelfth siphoning. Swing it to and fro in the air for a few moments to remove the ether adhering to it. By means of a pipette carefully run 5 c.c. of milk of known specific gravity on it,* taking care that all the milk is absorbed by the paper. The operation should be carried out over a clock glass, and any milk which drips from the paper should be re-absorbed by wiping it up with the roll. Suspend the roll in a beaker by means of a glass rod placed across the top, and place the beaker and roll in the water oven for an hour to dry.

The ether which has been used for the preliminary extraction should be dried by shaking in a dry separating funnel with granular calcium chloride and then distilled, the distillate being collected in a *dry* receiver. The flask B is then cleaned, dried, and weighed.

The extraction apparatus is again fitted up, and the roll containing the dried milk placed in the extraction tube. The dried, redistilled ether is poured through the condenser, the flask re-heated, and the extraction continued until twelve siphonings have taken place. All the fat contained in the milk will now be dissolved in the ether in the flask B. The Soxhlet extraction tube should be disconnected from the flask when it is nearly full of ether. This will save time in the subsequent distillation. The ether in the Soxhlet tube should be poured into a bottle labelled "ether residues."

The ether in the flask B is now distilled off as in the previous determinations, the flask being dried and weighed as before. From the increase in weight of the flask, calculate the percentage by weight of fat in the milk.

Example.—

Five c.c. of milk of specific gravity 1032 gave 0·1732 gm. of fat.

$$\text{Weight of milk} = 5 \times 1{\cdot}032$$
$$= 5{\cdot}160 \text{ gms.}$$

5·16 gms. of milk contained 0·1732 gm. of fat.

$\therefore$ 100 gms. of milk contained $\frac{0{\cdot}1732 \times 100}{5{\cdot}16}$ gms. of fat

$$= 3{\cdot}36 \text{ per cent.}$$

* Instead of measuring the milk by volume a known weight may be employed as in the previous determinations.

Calculation of the Extent to which Milk has been Adulterated.

Having determined the percentages of total solids and fat, it is obvious at once whether or not a sample of milk conforms to the legal standard.

The percentage of solids not fat is the difference between the percentages of total solids and fat. If a value less than 8·5 per cent. is found for the non-fatty solids, or less than 3 per cent. for the fat, it is concluded that the milk has been adulterated. This adulteration may be due to the addition of water or removal of cream, or both. The percentage of added water is calculated from the percentage of non-fatty solids.

Suppose, for example, a sample of milk contained 7·5 per cent. of non-fatty solids.

8·5 parts of non-fatty solids correspond to 100 parts of genuine milk.

$\therefore$ 7·5 ,, ,, ,, ,, $\frac{100 \times 7{\cdot}5}{8{\cdot}5}$,, ,,

$= 88{\cdot}2$,, ,,

Or in 100 parts of the milk under examination 88·2 parts are genuine milk and 11·8 parts are water.

Or percentage of added water = 11·8.

The undiluted milk would contain as a *minimum* 8·5 per cent. of non-fatty solids, so that *at least* 11·8 per cent. of water has been added.

The percentage deficiency of fat is calculated as follows :—

Suppose a given sample of milk contains 2·6 per cent. of fat.

On 3 parts of fat the deficiency is $3 - 2{\cdot}6 = 0{\cdot}4$.

$\therefore$ on 100 ,, ,, ,, $\frac{0{\cdot}4 \times 100}{3} = 13{\cdot}3$.

That is, the milk is deficient in fat to the extent of 13·3 per cent. ; or, of the three parts of fat which should be present in milk, it is assumed that 13·3 per cent. has been abstracted.

It should be noted in this case also that the milk before skimming may have contained more than 3 per cent. of fat, so that 13·3 per cent. is the minimum amount which has been abstracted. The standard of 3 per cent. of fat to which milk in this country must conform is considerably lower than that of other countries.

It will be obvious that the addition of water to milk will diminish the percentage of fat as well as the non-fatty solids, so that if the percentages of these constituents are less than 3 and 8·5 respectively, an allowance must be made for the deficiency of fat due to the addition of water before that due to removal of cream is calculated.

For example, a sample of milk was found to contain 7·5 per cent. of non-fatty solids and 2·6 per cent. of fat. The percentage of added water calculated from the non-fatty solids is 11·8 (see p. 10), so that 100 — 11·8, or 88·2 parts of the milk before the addition of water, contained 2·6 parts of fat.

∴ 100 parts of milk before the addition of water contained—

$$\frac{2{\cdot}6 \times 100}{88{\cdot}2} = 2{\cdot}95 \text{ parts of fat.}$$

So that on 3 parts of fat the deficiency due to removal of fat is $3 - 2{\cdot}95 = 0{\cdot}05$.

∴ on 100 parts of fat the deficiency due to removal of fat is $\frac{0{\cdot}05 \times 100}{3} = 1{\cdot}7$.

The sample thus contained 11·8 per cent. of added water, and was deficient in fat to the extent of 1·7 per cent.

DETERMINATION OF THE TOTAL PROTEIN IN MILK.

The total protein in milk is calculated from a determination of total nitrogen by the Kjeldahl method. In this process a weighed amount of the food is heated with concentrated sulphuric acid, containing potassium sulphate to raise its boiling-point and so diminish volatilisation, until a clear light yellow solution is obtained. The nitrogen contained in the substance is converted into ammonium sulphate. The carbon and hydrogen are oxidised to carbon dioxide and water by the sulphuric acid, which is reduced to sulphur dioxide. On distillation with sodium hydroxide the ammonia which is evolved is passed into a measured volume of a standard solution of sulphuric or hydrochloric acid. The excess of this acid, which is not neutralised by the ammonia, is determined by titration with a standard solution of sodium hydroxide. Hence the amount of ammonia produced is found, and from this the nitrogen contained in the amount of food taken. The percentage of nitrogen multiplied by a factor (6·39 in the case of milk) gives the percentage of protein.

Proteins contain on the average approximately 16 per cent. of nitrogen, so that percentage of nitrogen $\times \frac{100}{16}$ = per cent. of protein (see p. 154).

Approximately 5 gms. of milk (weighed accurately as described on p. 6) are placed in a Kjeldahl flask (Fig. 5), 5 gms. of powdered potassium sulphate are added, and 20 c.c. of concentrated sulphuric acid (nitrogen free) poured gradually into the mixture. Before the acid is added it is safer to evaporate the milk to dryness and cool the residue. It will, however, save time if the acid is added to the liquid, but great care must be taken to add the acid slowly and mix the liquid thoroughly after each addition of acid.

The flask is now supported on a wire gauze, as shown in the diagram, and gently heated by means of a Bunsen burner in a draught cupboard.

The flask must be heated gently at first to avoid frothing.

The heating is continued, the acid being allowed to boil gently, until a pale yellow solution is obtained. This will probably take about three hours. The flask is then allowed to cool.

If a small drop of mercury, or about 0·2 gm. of copper sulphate, be added to the mixture before heating, the time required for the completion of this part of the process will be considerably diminished. If mercury is employed, sodium or potassium sulphide solution must be added before the second (or distillation) part of the process is started, in order to decompose any ammoniacal mercuric compounds which are produced, as these are not decomposed by sodium hydroxide. In the presence of copper sulphate the solution obtained will be blue, and the heating should be continued for about half an hour after a clear solution is obtained.

The contents of the Kjeldahl flask are now diluted carefully with water and transferred to the flask A (Fig. 6), which is a 1-litre round bottomed flask. The Kjeldahl flask is washed out several times with small quantities of water, the washings being added to the contents of the flask A, and more water added until this flask is about half full. A piece of litmus paper is then put into the liquid in the flask.

A small quantity of zinc dust is placed in the dropping funnel B, and then approximately 50 c.c. of a solution of sodium hydroxide containing 500 gms. of sodium hydroxide in 1 litre of water. The finest zinc dust must be employed,

or it will not pass through the tap of B. The object of adding zinc dust is to liberate hydrogen from the sodium hydroxide during the distillation, and thus prevent bumping. Fifty c.c. of decinormal sulphuric acid, measured by means of a pipette, are placed in the beaker C, the height of which is adjusted so that the end of the condenser just dips below the surface of the acid. It is important that the condenser should not dip deeply into the acid, otherwise if the flask A

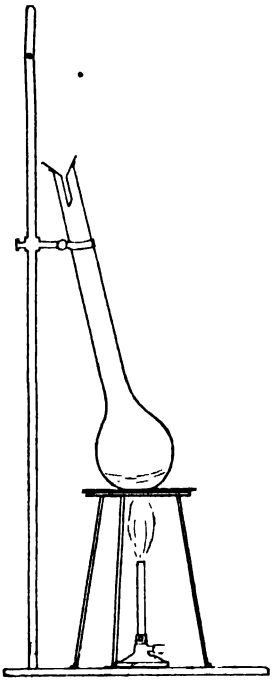


FIG. 5.—Kjeldahl Flask.

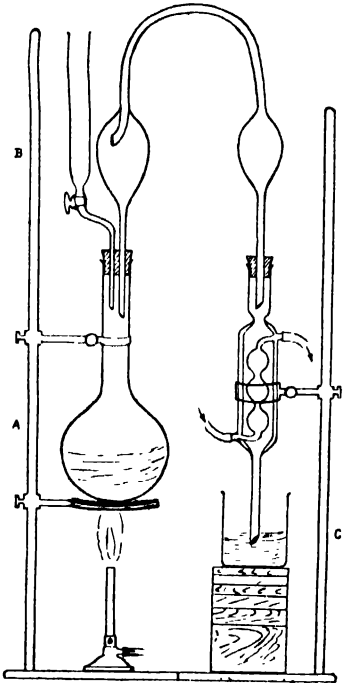


FIG. 6.—Distillation Apparatus.

is cooled (e.g. owing to a sudden draught) the standard acid in C may be sucked back into A. The sodium hydroxide solution in B is now allowed to drop slowly into the flask A, the contents of which are mixed by shaking. As soon as the sodium hydroxide mixes with the sulphuric acid in A, bubbling through the standard solution of sulphuric acid in the beaker C should take place, indicating that the apparatus is gas-tight. When all the sodium hydroxide from B has been added, the litmus paper in A, after thorough mixing of the contents,

should be blue. If this is not the case more sodium hydroxide should be added. The tap of B should now be closed, and the flask A heated so that the liquid boils gently. As the distillation proceeds lower the beaker C by removing some of the thin wooden blocks, so as to keep the end of the condenser only just beneath the surface of the standard solution of acid. Allow the distillation to proceed for at least half an hour and then remove the beaker C, wash the end of the condenser, and allow the washings to fall into this beaker. Test the distillate dropping from the end of the condenser for ammonia by means of litmus paper. If this liquid is free from ammonia the distillation is complete.

The excess of acid remaining in the beaker C is now titrated with decinormal sodium hydroxide solution, using methyl orange as indicator. From the amount of acid neutralised by the ammonia the percentage of nitrogen, and hence protein, in the milk is calculated as in the following example:

Weight of milk = 5·025 gms.

The ammonia was distilled into 50 c.c. of 1·1 N/10 H_2SO_4.

Volume of 1·2 N/10 NaOH required to neutralise excess of sulphuric acid = 27·9 c.c.

50 c.c. of 1·1 N/10 H_2SO_4 = 50 × 1·1 = 55 c.c. N/10 H_2SO_4

27·9 c.c. of 1·2 N/10 NaOH = 27·9 × 1·2 = 33·5 c.c. N/10 NaOH.

But 1 c.c. N/10 NaOH neutralises 1 c.c. N/10 H_2SO_4.

$\therefore$ 55 − 33·5 = 21·5 c.c. of N/10 H_2SO_4 were neutralised by the ammonia.

1 c.c. N/10 H_2SO_4 = 0·0049 gm. H_2SO_4
= 0·0017 gm. NH_3
= 0·0014 gm. N_2.

$\therefore$ 21·5 c.c. N/10 H_2SO_4 = 0·0014 × 21·5 = 0·0301 gm. of nitrogen.

$\therefore$ 5·025 gms. of milk contained 0·0301 gm. of nitrogen.

$\therefore$ 100 ,, ,, ,, $\frac{0{\cdot}0301 \times 100}{5{\cdot}025}$ gms. of nitrogen.

$$= \frac{0{\cdot}0301 \times 100 \times 6{\cdot}39}{5{\cdot}025} = 3{\cdot}83 \text{ gms. of protein.}$$

Hence the percentage of total protein = 3·83.

DETERMINATION OF LACTOSE IN MILK.

The approximate amount of lactose in milk may be calculated by deducting the sum of the percentages of fat,

proteins, and ash from the percentage of total solids, but a determination should be made of the amount of this substance in milk, either by means of Fehling's solution (*a*) or by means of the polarimeter (*b*). In both cases, however, it is necessary to precipitate the proteins before the determination of the lactose is carried out.

(*a*) DETERMINATION OF LACTOSE IN MILK VOLUMETRICALLY BY MEANS OF FEHLING'S SOLUTION.

Place 10 c.c. of milk of known specific gravity in a 100 c.c. graduated flask. Add 50 c.c. of distilled water and 2 c.c. of a 15 per cent. solution of potassium ferrocyanide, followed by 2 c.c. of a 30 per cent. solution of zinc acetate. Shake up the contents of the flask, add 3 drops of phenol-phthalein solution, and then add sodium hydroxide solution *drop by drop* until the liquid is *just* alkaline. Dilute the solution to 100 c.c., mix thoroughly, allow to settle, and filter through a dry filter. The filtrate which contains the lactose should be quite clear, the fat having been removed by the precipitated protein. The presence of lactose in this solution may be demonstrated by the preparation of lactosazone (see p. 70).

The object of diluting the milk is to obtain a solution of lactose which does not contain more than 1 per cent. of the substance, as with more concentrated solutions the method here described does not yield accurate results.

Place the lactose solution in a burette and measure out by means of a pipette 5 c.c. of No. 1 Fehling's solution (see p. 73) into a 4-inch porcelain basin or into a conical flask. Add 5 c.c. of No. 2 Fehling's solution and 30 c.c. of distilled water. Heat the basin until the solution begins to boil. Run in the lactose solution about 2 c.c. at a time, and boil the liquid between each addition, stirring with a glass rod. When only a faint blue colour remains, the lactose solution should be added in smaller quantities. To ascertain if the whole of the copper salt has been reduced to cuprous oxide, filter off a few drops of the liquid (using a very small filter paper without funnel), allowing the filtrate to fall on a white-glazed tile or a filter paper. Acidify the filtrate with acetic acid, and add 1 drop of a freshly-prepared, dilute solution of potassium ferrocyanide. If a brown coloration due to cupric ferrocyanide, $Cu_2Fe(CN)_6$, is obtained the titration should be continued until a test portion ceases to give this reaction.

Repeat the titration twice and take the mean of the last two readings. From the relation 10 c.c. of Fehling's solution (5 c.c. No. 1 + 5 c.c. No. 2) = 0·0676 gm. of lactose monohydrate, $C_{12}H_{22}O_{11} . H_2O$, or 0·0642 gm. of anhydrous lactose, $C_{12}H_{22}O_{11}$, it is possible to calculate the percentage of lactose in the original milk.

Example.—

Ten c.c. of milk of specific gravity 1032, after treatment with 2 c.c. of a 15 per cent. solution of potassium ferrocyanide, 2 c.c. of a 30 per cent. solution of zinc acetate, and sodium hydroxide solution till alkaline, were diluted to 100 c.c., the solution well mixed and filtered.

Volume of this lactose solution required for 5 c.c. No. 1 Fehling's solution + 5 c.c. No. 2 Fehling's solution + 30 c.c. of water was 17·5 c.c.

17·5 c.c. of the solution = 1·75 c.c. of original milk (i.e. 10 c.c. of milk were diluted to 100 c.c.).

1·75 c.c. of milk of specific gravity 1032 = 1·75 × 1·032 gm.
= 1·806.

∴ 1·806 gm. of milk contained 0·0642 gm. of anhydrous lactose.

∴ 100 gms. of milk contained $\frac{0{\cdot}0642 \times 100}{1{\cdot}806}$
= 3·56 per cent. of anhydrous lactose.

For details of a gravimetric process for the determination of lactose in milk by means of Fehling's solution, see "Dairy Chemistry," by Droop Richmond.

(*b*) DETERMINATION OF LACTOSE IN MILK BY MEANS OF THE POLARIMETER.*

In this determination the proteins and fat are first precipitated from a given volume of milk by means of an acid solution of mercuric nitrate, and the solution diluted to a known volume. For very exact determinations precipitation by acid mercuric nitrate should be followed by addition of phospho-tungstic acid, which causes the precipitation of a further small quantity of protein. The volume occupied by the precipitated proteins and fat is calculated, and a volume of water equal to this volume is added. The solution is then

* For a description of this instrument and its method of employment, see p. 81.

filtered and the rotation of the solution in a 2 dcm. tube observed.

The specific rotatory power of lactose monohydrate, $C_{12}H_{22}O_{11} . H_2O$, at 15° C., for the sodium flame, denoted $[\alpha]_D^{15° C.}$, is + 52·5°, so that by measuring the rotation produced by a known length of the solution the amount of lactose present may be calculated by the method described on page 92.

Place 60 c.c. of milk of known specific gravity in a 100 c.c. graduated flask and add 3 c.c. of acid mercuric nitrate solution. (This solution is made by dissolving mercury in twice its weight of nitric acid, of specific gravity 1·42, and adding a volume of water equal to that of the solution when all the mercury has dissolved.) Dilute the mixture of milk and mercuric nitrate to 100 c.c. with distilled water.

The volume occupied by the precipitated protein and fat must now be calculated.

For this purpose it may be assumed that 1 gm. of milk fat = 1·075 c.c., and 1 gm. of milk proteins = 0·8 c.c.

From the known percentages by weight of fat and protein in the milk under examination, calculate the *volume* of fat and protein in 60 c.c. of milk. Add a volume of water from a dropping pipette, or burette, to the mixture in the 100 c.c. flask equal to the volume of fat *plus* protein in the 60 c.c. of milk. Mix the contents of the flask thoroughly and filter through a dry filter paper. Reject the first portion of the filtrate if it is not perfectly clear. Measure the rotation of the solution in a 2 dcm. tube. Calculate the percentage of lactose monohydrate, and from this the percentage of anhydrous lactose in the original milk.

Example.—

60 c.c. of milk of specific gravity 1030 were treated with 3 c.c. of acid mercuric nitrate solution, and the mixture diluted to 100 c.c.

Percentage of fat in sample of milk 3·4 per cent. by weight.

,, ,, protein ,, ,, 3·3 ,, ,,

60 c.c. of milk = 60 × 1·03 = 61·8 gms.

Weight of fat in 61·8 gms. of milk

$$= 61·8 \times \frac{3·4}{100} = 2·1 \text{ gms.}$$

Volume of this fat = 2·1 × 1·075 c.c.
= 2·26 c.c.

Weight of protein in 61·8 gms. of milk

$$= 61{\cdot}8 \times \frac{3{\cdot}3}{100} = 2{\cdot}04 \text{ gms.}$$

Volume of this protein $= 2{\cdot}04 \times 0{\cdot}8$ c.c.
$= 1{\cdot}63$ c.c.

∴ Volume of water equal to the volume of fat *plus* protein to be added to 100 c.c. of the mixture $= 2{\cdot}26 + 1{\cdot}63$
$= 3{\cdot}9$ c.c.

3·9 c.c. of water were added to the mixture in the 100 c.c. flask, the flask was then stoppered and the contents thoroughly mixed by shaking.

Some of the mixture was then filtered through a dry filter paper, and a 2 dcm. polarimeter tube filled with the clear filtrate.

Zero reading of instrument $= + 0{\cdot}26°$, average of four readings.

Reading with solution of lactose $= + 3{\cdot}55°$, average of four readings.

Rotation due to lactose solution $= + 3{\cdot}29°$.

Rotation due to a length of 1 dcm. of solution would be

$$\frac{+ 3{\cdot}29}{2} = + 1{\cdot}65°.$$

But $+ 52{\cdot}5°$ is the rotation for 1 dcm. of a solution containing 100 gms. of lactose monohydrate in 100 c.c.

∴ $+ 1{\cdot}65°$,, ,, ,, $\frac{1{\cdot}65 \times 100}{52{\cdot}5}$,,
$= 3{\cdot}14$ gms. lactose monohydrate.

∴ 100 c.c. of the solution contained 3·14 gms. $C_{12}H_{22}O_{11} . H_2O$ or 61·8 gms. of milk contain 3·14 gm. $C_{12}H_{22}O_{11} . H_2O$.

∴ 100 ,, ,, $\frac{3{\cdot}14 \times 100}{61{\cdot}8}$
$= 5{\cdot}09$ gms. $C_{12}H_{22}O_{11}H_2O$.

But 360 gms. $C_{12}H_{22}O_{11} . H_2O = 342$ gms. of anhydrous lactose, $C_{12}H_{22}O_{11}$.

∴ 5·09 gms. $= \frac{5{\cdot}09 \times 342}{360} = 4{\cdot}85$ gms. ,, ,,

The sample of milk contained 4·85 per cent. by weight of anhydrous lactose.

DETERMINATION OF THE TOTAL ACIDITY OF MILK.

In this determination a measured volume of milk is titrated with N/10 sodium (or barium) hydroxide, using phenolphthalein as indicator. *The volume of N/10 alkali required for 100 c.c. of milk gives the so-called "degree of acidity"* of the milk.

Freshly drawn milk, though probably free from lactic acid, is usually acid to phenolphthalein, owing to the presence of carbonic acid, acid phosphates, etc. The total acidity of milk is, however, often expressed in terms of lactic acid. From the equation—

$$\underset{\substack{90\\ \text{lactic acid}}}{CH_3CH(OH)COOH} + \underset{40}{NaOH} \rightarrow CH_3CH(OH)COONa + H_2O$$

it will be seen that 40 gms. of sodium hydroxide, or 10,000 c.c. N/10 NaOH solution, is equivalent to 90 gms. of lactic acid, or 1 c.c. N/10 NaOH = 0·009 gms. of lactic acid.

The so-called *degree of acidity* of fresh milk usually varies from 15 to 18. An acidity of 20° to 22° is perceptible to the taste, and if the acidity is much in excess of this amount the milk will coagulate on heating.

Measure out 20 c.c. of milk into each of two conical flasks (or boiling tubes), and add 1 c.c. of a 0·5 per cent. solution of phenolphthalein in 50 per cent. alcohol. Add N/10 sodium hydroxide solution from a burette to the contents of one of the flasks until a permanent slight pink colour is obtained, after the contents of the flask have been mixed by shaking. The colour of the solution to which the sodium hydroxide is being added should be compared with that of the milk used as control, and the titration stopped as soon as the faintest permanent pink colour is obtained. Repeat the titration twice more, and take the mean of the last two readings.

Calculate the volume of N/10 sodium hydroxide solution which would be required for 100 c.c. of the milk, and so get the *degree of acidity*.

Express the result also as grams of lactic acid per 100 c.c. of milk, and as percentage by weight.

The determination of the acidity of milk is of great importance in connection with the artificial souring of milk for use in the manufacture of margarine (p. 51).

From a bacteriological point of view the hydrogen ion concentration of milk is of more importance than the degree of acidity (see p. 179).

ADDED COLOURING MATTER IN MILK.

The colour of unadulterated milk is due to the milk fat, and it is, of course, a matter of common experience that the cream of such milk is deeper in colour than the milk below the cream layer. Previous to 1918 it was often found that milk in London and elsewhere contained added colouring matter, such colouring matter sometimes being added to mask the change in colour produced by the addition of water. By the Food Controller's order of 1918 this addition of colouring matter was made illegal.

The colouring matters most frequently employed are annatto—a reddish-yellow substance derived from the seed of a plant found in Central America and elsewhere—or an azo dye of the nature of methyl orange (see p. 147). Caramel (see p. 227) has sometimes been used, but is not very suitable for this purpose.

Test for added colouring matter in—(*a*) milk as supplied; (*b*) milk to which a small quantity of methyl orange (yellow solution) has been added; (*c*) milk to which a small quantity of a solution of annatto in dilute sodium hydroxide solution has been added. The tests should be carried out as follows:—

(1) Allow some of the milk to stand for several hours, or overnight, in a test tube. If colouring matter has been added the layer below the cream will probably be more highly coloured than the cream layer.

(2) Add solid sodium bicarbonate to some of the milk until it is alkaline to litmus, and place a strip of filter paper in the milk. If on standing overnight the paper shows a slight reddish yellow colour, annatto is present. Wash the paper under the tap for a few seconds and treat with stannous chloride solution. A pink stain confirms the presence of annatto.

(3) Add a small quantity of dilute hydrochloric acid to some of the milk. If methyl orange or a similar azo dye is present a pink colour is obtained. The colour of annatto is not changed by mineral acids.

It should be noted that although milk becomes acid on standing, the weak acid, lactic acid, which is produced does not give a pink colour with methyl orange owing to its low concentration of hydrogen ions, otherwise milk containing methyl orange would become pink on standing. On the other hand, it is sometimes found that margarine turns pink in a

chemical laboratory owing to the action of hydrochloric acid in the air of the room on the azo dye used as colouring matter.

For further information concerning added colouring matter, see " Dairy Chemistry," by Richmond, and " Food Inspection and Analysis," by Leach.

PRESERVATIVES IN MILK.

The addition of any substance as a preservative to milk (including fresh, skimmed, condensed, and dried milk) was prohibited under Public Health Regulations, 1912. The addition of 0·4 per cent. of boric acid, or a mixture of borax and boric acid, or the addition of hydrogen peroxide, to cream is allowed under certain conditions at the present time. It is possible, however, that the addition of any preservative to cream will be made illegal.

The preservatives most commonly used in connection with milk are boric acid and borax, or formaldehyde. In connection with the use of formaldehyde as a preservative for milk, it should be noted that whilst this substance retards the multiplication of the lactic acid forming bacilli, and so delays souring of the milk, it does not prevent the multiplication of other organisms such as tubercle bacilli, which are harmful. It has been said that " the man (or woman) who adds formaldehyde to milk takes down the danger signal, but does not remove the danger."

In carrying out the tests described below, use (*a*) milk as supplied; (*b*) milk containing about 0·1 gm. of boric acid in 100 c.c.; (*c*) milk containing one drop of " formalin " (40 per cent formaldehyde) solution in about 100 c.c. of milk.

TESTS FOR BORIC ACID AND BORAX.

Place approximately 1 c.c. of milk in a porcelain basin, such as is used for the determination of total solids, add one drop of concentrated hydrochloric acid, and mix by means of a glass rod. Now add about six drops of a saturated alcoholic solution of turmeric.* Evaporate to dryness by heating gently on a water bath, or on the top of a hot water oven. In the presence of boric acid or borax a pink colour develops. If

* Made by boiling turmeric with alcohol for an hour under a reflux condenser, allowing to cool, and filtering. It is important that the turmeric solution should be prepared in this way.

the residue is heated too strongly a brown coloration is produced. This test is extremely delicate, and as little as 0·02 per cent. of boric acid may be detected by means of it.

Other tests for the presence of borax and boric acid may be made on the ash resulting from the ignition of the residue obtained by heating milk made alkaline with sodium hydroxide to prevent loss of boric acid (see also p. 165). The tests described in Volume I., page 35, with turmeric and with sulphuric acid and alcohol may be applied to this ash.

DETERMINATION OF BORIC ACID (AND BORAX) IN MILK.

In the method usually employed for this determination the boric acid is titrated with a standard solution of sodium hydroxide, after removal of carbonic and phosphoric acids, the final titration being carried out in presence of glycerol to prevent hydrolysis of the sodium metaborate (see Vol. I., p. 40). This method necessitates ignition of the residue obtained by evaporation of the milk to dryness and other operations, which take a considerable time. A simpler empirical method, which gives fairly accurate results, has been devised by Richmond and Miller, and is carried out as follows :—

To 10 c.c. of milk of known specific gravity add 5 c.c. of 0·5 per cent. solution of phenolphthalein. Add sodium hydroxide solution until a pink coloration is obtained. Boil the mixture and add dilute hydrochloric acid to the boiling solution until the colour is discharged. While still boiling gently, add N/10 sodium hydroxide solution from a burette until a *faint* pink colour is obtained. Discontinue heating and add glycerol to the mixture equal to approximately half its volume, and continue the addition of N/10 sodium hydroxide until a pink colour is obtained. The volume of N/10 sodium hydroxide required after the addition of the glycerol gives a measure of the amount of boric acid in 10 c.c. of the milk. From this the amount of boric acid in 100 gms. of the milk is calculated. From the equation—

$$H_3BO_3 + NaOH \rightarrow NaBO_2 + 2H_2O$$

it will be seen that

1 c.c. N/10 NaOH = 0·0062 gm. H_3BO_3.

(See Vol. I., p. 40.)

It should be noted that although borax and not boric acid may have been added to the milk, the result is expressed in terms of boric acid.

TESTS FOR FORMALDEHYDE IN MILK.

Hehner's Test.—Place from 5 to 10 c.c. of milk in a test tube and carefully pour down the side of the tube about half as much concentrated *commercial* sulphuric acid, to form a layer at the bottom. If formaldehyde is present a violet ring is produced at the junction of the two liquids.* This test depends on the presence of traces of iron compounds in the acid, and this is the reason for not using the *pure* acid.

It should be noted that if excess of formaldehyde is present the violet coloration is not produced. It is therefore advisable to test the milk before and after dilution with water. One part of formaldehyde in 200,000 of milk may be detected by means of this test.

Hydrochloric Acid and Ferric Chloride Test.—Milk containing formaldehyde heated with three to five times its volume of concentrated commercial hydrochloric acid (which will contain ferric chloride) gives a violet coloration.

DETERMINATION OF FORMALDEHYDE IN MILK.

Liverseege's Method.—10 c.c. of the milk are placed in a wide test tube and concentrated sulphuric acid, containing a few drops of ferric chloride solution, is added, about 1 c.c. at a time, shaking after each addition, until about 6 c.c. have been added. The purple colour obtained is compared with that obtained with samples of pure milk, to which known amounts of a dilute, standard solution of formaldehyde have been added. In this way the amount of formaldehyde in the sample is found.

"MYSTIN."

A solution of formaldehyde (about 0·3 per cent.) containing 10 per cent. of sodium nitrite is sold under the above name. The presence of the nitrite prevents the detection of formaldehyde by the usual tests. The nitrous acid probably interacts with tryptophane, one of the products of hydrolysis of casein,

* This coloration may be observed in the Gerber test (p. 4) if the milk contains formaldehyde.

and the tryptophane is thereby rendered incapable of giving a coloration with the formaldehyde.

The nitrite may, however, be removed from such preparations by treatment with urea and sulphuric acid, and the milk then tested for the presence of formaldehyde by Hehner's test.

To 5 c.c. of a sample milk containing formaldehyde and sodium nitrite * add about 0·05 gm. of urea (2·5 c.c. of 2 per cent. urea solution) and 1 drop of dilute sulphuric acid. Heat in boiling water for about two minutes, *cool*, and add commercial sulphuric acid (see p. 23).

A purple ring at the junction of the liquids indicates the presence of "mystin."

If no formaldehyde was found in the original sample of milk, the presence of this substance should be tested for after the addition of urea and sulphuric acid as described above.

Other preservatives sometimes used are benzoic or salicylic acid, or hydrogen peroxide. The detection of these substances when used as preservatives in other foods is dealt with on pages 186 and 26.

BOILED AND PASTEURISED MILK

Fresh milk contains enzymes of the "peroxydase" type, i.e. enzymes which have the power of transferring oxygen from a peroxide, such as hydrogen peroxide, to an oxidisable substance. The function of these enzymes is destroyed if the milk has been heated above 80° C. (see p. 104).

In carrying out the test described below use (*a*) fresh milk, (*b*) boiled milk.

1. Allow samples of each to stand and note that in the case of the boiled milk the cream rises very slowly.

2. Treat 5 c.c. of the milk in test tubes with 2 drops of a 2 per cent. solution of para-phenylene diamine, $C_6H_4(NH_2)_2$, and add 1 drop of a very dilute solution of hydrogen peroxide (10 volume solution diluted 1 in 50).

In the case of the unheated milk a dark bluish violet colour is produced immediately, whereas in the case of the heated milk no colour is produced for some time.

For further information on this subject, see "Milk and Its Hygienic Relations," by Lane-Claypon, and "Fatty Foods,"

* This sample may be prepared as follows: To 100 c.c. of the milk containing 1 drop of formalin, 1 c.c. of 3 per cent. sodium nitrite solution is added.

by Bolton and Revis. For other changes produced by heating milk, see page 232.

CREAM.

The chief difference between milk and cream is, of course, that in the latter the percentage of fat is usually much higher than in the former. Great variations are, however, found in the percentage of this constituent in various samples of cream. Some samples may contain 25 per cent. of milk fat, and others nearly 60 per cent. of this constituent. The percentage of non-fatty solids is usually less than 7.

Addition of 0·4 per cent. of boric acid as preservative, or addition of hydrogen peroxide is allowed under Public Health (Milk and Cream) Regulations if the cream contains not less than 35 per cent. of milk fat. Such cream must, however, be described as "preserved cream," and the amount of boric acid used must be specified.*

A measure of the percentage amount by volume of "cream" (not, of course, milk fat) in milk may be obtained by allowing 100 c.c. of milk to stand in a 100 c.c. graduated cylinder and reading off the volume of the cream layer.

The percentage of milk fat in cream is determined by diluting a known weight of the cream to a definite volume with distilled water and determining the fat in a known volume of the mixture by the Rose-Gottleib or Werner-Schmidt method. If the Adams method is employed, a known weight of the cream is dried on the coil of filter paper before extraction.

Any other constituent of the cream may be determined by the methods described under milk.

*Artificial Thickening of Cream.**—Gelatinised starch, gelatine, and "viscogen" (a solution of cane sugar in lime water) have been employed for this purpose. The last-named is the most effective. Starch is easily detected by the production of the well-known blue colour, when a mixture of the cream and water is treated with a dilute solution of iodine in potassium iodide. To test for gelatine, to a mixture of about 5 gms. of the cream and 10 c.c. of water, add 1 c.c. of acid mercuric nitrate solution (see p. 17) to precipitate proteins and remove the fat. Shake well and filter. To the filtrate add saturated aqueous solution of picric acid, a yellow precipitate is produced in presence of gelatine.

* It is probable that shortly it will be illegal to sell cream to which either preservative or thickening substance has been added. See Draft Rules, Note p. 149.

Cane sugar may be detected as follows: Add to a mixture of approximately 5 c.c. of cream and 10 c.c. of water in a test tube about 0·5 gm. of powdered ammonium molybdate and 5 c.c. of dilute hydrochloric acid, and heat the test tube in a water bath to 80° C. to 90° C. If cane sugar is present in the cream a blue colour is produced. Cream or milk free from cane-sugar treated in this way give a colour only on *boiling*.

The addition of condensed milk to cream would lead to a high percentage of non-fatty solids.

PRESERVATIVES IN CREAM.

The presence of preservatives in cream may be tested for and the amount present determined by the methods employed for milk. For the determination of boric acid 10 gms. of the cream mixed with 10 c.c. of water may be employed.

Hydrogen peroxide may be tested for by means of p-phenylene diamine (p. 24). It is probable, however, that if hydrogen peroxide has been added to the cream most of it will have decomposed before the cream is examined for its presence.

Sodium fluoride is sometimes used as a preservative, and this may be detected by treating the ash with concentrated sulphuric acid and examining for hydrofluoric acid by the etching of glass test.

If borate and fluoride are present together a special procedure is necessary. (See Allen, "Commercial Organic Analysis," Vol. 8.)

CONDENSED MILK.

In the preparation of this product milk is evaporated to about one-third of its bulk. The evaporation of the heated milk is usually effected under reduced pressure, the temperature being kept as low as possible in order that the final product shall not have the characteristic taste of boiled milk. Cane sugar is added in some varieties, and is often present to the extent of 40 per cent. of the product. In addition to its use on account of its sweetening properties, cane sugar acts as a preservative.

It should be noted that condensed milk is made from skimmed milk as well as from whole milk, and if made from the former this must be specified on the label. Sweetened condensed milk usually contains many micro-organisms, but the presence of the cane sugar prevents their multiplication. Condensed skimmed milk, on the other hand, is often sterile.

The question as to the standard to which condensed milk should conform has recently been put on a more satisfactory basis. Under the Public Health (Condensed Milk) Regulations, 1923, standards for condensed milk are laid down as follows:—

	Percentage of milk fat not less than	Percentage of all milk solids, including fat not less than
1. Full cream, unsweetened	9·0	31·0
2. Full cream, sweetened	9·0	31·0
3. Skimmed, unsweetened	—	20·0
4. Skimmed, sweetened	—	26·0

In addition, definite regulations as to labelling each of the four brands have been made.

For analysis of condensed milk a weighed amount, 10 to 20 gms., of the material is made up to 100 c.c. with water, and the various constituents determined as described under milk. The percentage of cane sugar in sweetened condensed milk is determined by difference, by deducting the sum of the percentages of protein, lactose, fat, and ash from the percentage of total solids. It may, however, be determined directly by means of Fehling solution after inversion, a deduction being made for the lactose present, as in the determination of sucrose and invert sugar (see p. 113).

For further information on the subject of condensed milk, see Local Government Board Food Report, No. 15, and Report of the Food Investigation Board, No. 13, by Savage (H.M. Stationery Office, 1923).

DRIED MILK POWDERS.

These preparations are made by evaporation of water from whole or skimmed milk by either a "*roller*" or a "*spray*" process. In one form of roller process the milk is allowed to fall on revolving cylinders, mounted horizontally and heated internally by steam under pressure. The temperature of the roller is thus above 100° C. The milk rapidly loses water, and the milk solids are immediately scraped off the roller. In another roller process the milk which has been condensed, and possibly sterilised, is taken up on the outside of a revolving cylinder filled with hot water. The upper portion of the roller is fitted with a loosely fitting cover, by means of which air

can be drawn over the milk layer and evaporation facilitated. The milk solids are removed from the roller as before.

In the spray process pasteurised condensed milk, in the form of a very fine spray, is forced by means of a jet of dry air at 115° C. into a chamber through which hot air circulates. In this way the milk solids are obtained in a very fine state of division, and dried milk obtained by this process is more easily incorporated with water to form a homogeneous mixture than is the case with dried milk prepared by a roller process. For this reason spray process dried skimmed milk is more suitable than a roller process dried milk for use in connection with the manufacture of margarine.

It should be noted, however, that spray process dried milk is in contact with hot air for a longer period than is the case with milk dried by a roller process. This is probably of some importance in connection with the question of the presence of accessory factors in the final product (see p. 224).

In most cases no addition of any substance is made to the milk, if quite fresh, which is employed in the production of dried milk, but sodium bicarbonate or an alkaline phosphate is sometimes added to neutralise the acidity of the milk and prevent curdling when the milk comes in contact with the hot roller. If curdling takes place it is difficult to remove the solid. Probably most of the dried milk used at the present time is imported in the solid form, but a certain amount of dried milk is prepared in this country by the spray process.

For further information concerning the preparation of milk powders, see Local Government Food Report, No. 24.

EXAMINATION OF DRIED MILK.

The chief difficulty associated with the use of dried milk is met with in the production of a homogeneous mixture with water, and unless the powder is properly incorporated in the mixture large fat globules and particles of casein separate on standing. This is overcome, however, by the use of an efficient emulsifier, and an electrically driven apparatus for this purpose is often employed where large quantities of dried milk have to be dealt with.

Milk obtained by mixing dried milk powder with water, both by hand and by means of an emulsifier, should be examined under the microscope. In the latter case, it will be seen that the fat globules are of approximately the same size as in ordinary milk.

In analysing milk powders it is usual to calculate the composition of the milk employed for the production of the powder. To do this, what is known as the *concentration factor* must be determined. The ash content of average whole milk is 0·75 per cent., and if in a milk powder 6·0 per cent., the ash has been increased $\frac{6{\cdot}0}{0{\cdot}75} = 8$ times. So that if nothing has been added to the milk and only water abstracted in the production of the milk powder, 8 parts by weight of milk have been evaporated to produce 1 part by weight of milk powder, or the concentration factor in this case is 8. Or, since the sum of the percentages of protein and lactose in whole milk is on the average 8·15 per cent., the sum of the percentages of protein and lactose in the milk powder divided by 8·15 also gives the concentration factor. The latter method is used if there is reason to believe that inorganic matter has been added to the milk.

Dried milk powders made from whole and skimmed milk usually contain :—

	Whole Milk, per cent	Skimmed Milk, per cent.
Moisture . . .	3·5 to 5	5 to 7
Fat	25 ,, 28	1·5 ,, 2
Protein . . .	23 ,, 25	31 ,, 34
Lactose (hydrated) .	35 ,, 37	47 ,, 52
Ash . . .	6 ,, 6·5	7·5 ,, 8·5
Concentration factor .	7 ,, 8	9·5 ,, 10

Moisture and Ash can be determined on 1 gm. of the powder by drying at 100 °C. to constant weight and subsequently incinerating.

Fat is determined by the Werner-Schmidt process (p. 7), using 1 gm. of the powder mixed with about 8 c.c. of water. A slightly low result is obtained by the Gottleib-Rose method, and it is very difficult to extract the fat completely by the Soxhlet extraction method.

Protein is determined by the Kjeldahl process, using 1 gm. of the substance (see p. 11).

Lactose is usually determined *gravimetrically* by the Fehling solution process, but if the mixture has been properly emulsified the *volumetric* method described on page 15 may be employed.

The addition of alkali or phosphate is shown by an excess of these substances in the ash of dried milk. Milk powder in a dry condition is not a suitable medium for the growth of bacteria, and preservatives are very rarely found in these products. Colouring matters also, as a rule, are not employed in their manufacture.

For further information on this subject, see Local Government Food Report, No. 24, p. 177, and a paper by Jephcott, "The Analyst," 1923, p. 529.

Chapter II.

EDIBLE OILS AND FATS.

GENERAL CHARACTERISTICS.

These substances, which are of both animal and vegetable origin, are mixtures of glycerides derived mainly from fatty acids of the general formula $C_nH_{2n+1}COOH$, which have an *even* number of carbon atoms in the molecule, i.e. an *odd* number of carbon atoms in the radical which is combined with the carboxyl group. (See table below.) Glycerides derived from *unsaturated* acids such as oleic acid, $C_{17}H_{33}COOH$, linoleic acid, $C_{17}H_{31}COOH$, etc., are also of importance in connection with edible oils and fats, and in some cases derivatives of hydroxy acids are found.

The chemical nature of these glycerides has been dealt with in connection with soap (Vol. I., Chap. III.).

It should be noted that the distinction between an edible oil and a fat is simply due to differences in physical state. Mixtures of glycerides which are solid at the ordinary temperature are usually referred to as fats, and those which are liquid as oils. It is perhaps advisable to point out that although all edible oils are saponifiable oils, *all* substances described as oils are not saponifiable substances. Thus paraffin oil, olive oil, and oil of turpentine are names given to mixtures; in the first and last cases to mixtures of hydrocarbons, and in the second case to a mixture of glycerides.

Glycerides in natural edible oils and fats derived from the various fatty acids are met with as follows:—

Acid, $C_nH_{2n}H_{+1}COOH$		Glyceride, $C_3H_5(OOC.C_nH_{2n+1})_3$ occurs in
Butyric acid,	C_3H_7COOH	Butter.
Caproic	$C_5H_{11}COOH$	Butter, coconut, and palm oils.
Caprylic ,,	$C_7H_{15}COOH$	
Capric ,,	$C_9H_{19}COOH$	,, ,, ,,
Lauric ,,	$C_{11}H_{23}COOH$	Coconut and palm oils.
Myristic ,,	$C_{13}H_{27}COOH$	Butter, coconut, and palm oils.
Palmitic ,,	$C_{15}H_{31}COOH$	Nearly all oils and fats.
Stearic ,,	$C_{17}H_{35}COOH$	,, ,, ,,
Arachidic ,,	$C_{19}H_{39}COOH$	Peanut (arachis) oil.

As the number of carbon atoms increases, the melting-points of the acids and glycerides derived from them are raised. The acids of low molecular weight of the above series are volatile when distilled with steam, and are soluble in water, the higher acids of the series are only very slightly volatile in steam, and are insoluble in water. This is of importance in connection with the analysis of a mixture of glycerides (see p. 39).

Glycerides derived from oleic acid, an unsaturated acid of the formula $C_{17}H_{33}COOH$ and other unsaturated acids, are present in nearly all edible oils and fats. These glycerides have lower melting-points than the glycerides derived from the corresponding saturated acids, into which they are converted by an important process known as *hydrogenation* (see p. 45). A natural fat containing a relatively high proportion of unsaturated glyceride has thus a low melting-point.

Glycerides derived from more unsaturated acids, such as linolic acid, $C_{17}H_{31}COOH$, linolenic acid, $C_{17}H_{29}COOH$, and glycerides derived from unsaturated acids of the formulæ $C_{17}H_{27}COOH$ and $C_{21}H_{33}COOH$ are found in fish oils.

It is important to realise that any natural edible oil or fat is a complex mixture of glycerides, and the analysis of these substances is much more complicated than if we were concerned with single compounds.

The mixtures of glycerides with which we are chiefly concerned are butter fat (i.e. milk fat) and the fat present in margarine, which may be of animal or vegetable origin. In the latter case the substances of importance are coconut, palm kernel, arachis, and cotton-seed oils. Reference will also be made to olive oil, lard, and cheese. Linseed oil, although not an edible oil, is of the same chemical nature as these substances, and the properties of unsaturated glycerides will be illustrated by reference to this oil.

PHYSICAL AND CHEMICAL CHARACTERISTICS.

PHYSICAL CHARACTERISTICS AND PROCESSES.

Filtration and Weighing of Fats and Oils.—It is often necessary to free an oil or melted fat from suspended matter. This is effected by filtration, but in the case of a fat solidification in the stem of the funnel takes place and the process comes to an end. This is obviated by employing a hot water

jacketed funnel as shown in the diagram (Fig. 7). In using this apparatus it is important to see that the water in the copper vessel does not boil away completely.

Oils and fats are usually weighed out for experiments by weighing some of the substance in a small beaker, containing a short glass rod, pouring out a portion of the substance, and re-weighing. The amount taken being found by difference. In the case of a fat the substance is poured out in the liquid condition and allowed to solidify before re-weighing.

Solvents for Fats.—Glycerides are insoluble in water, slightly soluble in alcohol, and readily soluble in ether, benzene, chloroform, carbon tetrachloride, and other chlorine substitution products of hydrocarbons. (See Vol. I., Chap. VI.)

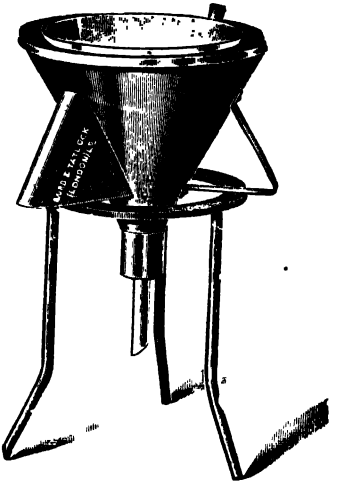


FIG. 7.—Hot Water Funnel.

The extraction of fat from a mixture by means of ether has been described under milk. This Soxhlet extraction of fat by means of ether or light petroleum is very largely made use of in determining the amount of fat in a dry substance, and for obtaining fat in solution free from other materials. It must be noted, however, that substances other than fats are soluble in ether, and fat extracted in this way may contain unsaponifiable substances. It should also be noted that in certain cases it is necessary to remove the solvent from an ethereal solution of fat in a current of carbon dioxide, to prevent absorption of oxygen by the fat.

The Melting-point and Solidifying-point of Oils and Fats.—The solidifying-point is determined as described for paraffin wax (Vol. I., p. 258). The melting-point of a fat may be determined by the capillary tube method, the fat being contained in a capillary tube open at both ends. In this method a capillary melting-point tube, open at both ends, is dipped into the melted fat to the depth of about 1 cm. and then withdrawn. The fat solidifies on cooling, and the tube is cleaned on the outside. The capillary tube attached to a

thermometer is then heated *slowly* in a beaker of *water*, and the temperature noted at which the fat melts.

It is usually found that the melting-point of a fat is higher than the solidifying-point.

The determination of the solidifying-point of the acids obtained from fats by hydrolysis (the Titer test) is of importance in connection with the analysis of these substances.

The Specific Gravity of an Oil.—This may be determined by the specific gravity balance (see "Chemistry of Petroleum.")

The specific gravities of edible oils at 15° vary between 0·91 and 0·94 (water at 15° C. = 1), and it is obvious that little, or no, information as to the composition of an oil can be obtained by this determination, owing to the fact that by suitable admixture of oils a liquid having any specific gravity between 0·91 and 0·94 may be obtained.

Solid fats have specific gravities between 0·86 and 0·87.

Refractive Index.—Oils and fats vary considerably in refractive index, and the determination of this constant is often of great value in connection with oil analysis. Various forms of apparatus for this determination are in use, and a description of each particular form of refractometer and the method of using it is usually supplied by the maker of the instrument.

CHEMICAL CHARACTERISTICS AND PROCESSES.

The Saponification Value of an Oil or Fat.

The number of *milligrams* of potassium hydroxide required for the saponification of 1 gm. of an oil or fat is known as its *saponification value* or *saponification number*. The determination of this value is of great importance in connection with the analysis of these substances.

It will be seen from the equation given below that for the saponification of 1 gm. molecule (806 gms.) of tripalmitin, 3 gm. molecules (168 gms.) of potassium hydroxide are required. From this it is calculated that 1 gm. of tripalmitin requires 0·208 gms., or 208 milligrams, of potassium hydroxide for saponification. That is, the *saponification value* of pure tripalmitin is 208.

$$\begin{array}{lll} CH_2|\overline{OOCC_{15}H_{31}} & K|OH & CH_2OH \\ CH\ |OOCC_{15}H_{31} & K|OH \rightarrow 3C_{15}H_{31}COOK + CHOH & \\ \quad | & \text{Potassium palmitate.} & | \\ CH_2|OOCC_{15}H_{31} & K|OH & CH_2OH \\ \text{Tripalmitin.} & & \text{Glycerol.} \\ 806 & 3 \times 56 & \end{array}$$

Similarly, it can be calculated that the saponification value of tributyrin is 485, of tristearin 188·8, and of triolein 190.

For natural oils and fats, which are mixtures of glycerides, the saponification value will therefore depend on the relative proportions of the various glycerides present in the mixture.

As might be expected for such mixtures, different samples of the same kind of edible oils and fats give slightly different values, and in the table given below the figures given are the extreme values for different samples.

Oil or Fat.	Saponification Value.
Arachis oil	190-196
Butter fat	220-240
Coconut oil	246-260
Cottonseed oil	192-195
Lard	193-203
Linseed oil	190-195
Mutton tallow	192-195
Olive oil	185-196
Palm kernel oil	245-248
Whale oil	188-194

It will be noticed that the saponification values for different oils in some cases are very similar, and it is obviously impossible to identify an oil simply by determining this value. The question of the saponification value of a mixture of two saponifiable oils, and of a mixture of a saponifiable and unsaponifiable oil is dealt with later (see p. 37).

Determination of the Saponification Value of an Oil.—A known weight of the oil is heated with a measured volume of a standard solution of alcoholic potash, until saponification is complete. The excess of potassium hydroxide remaining in the solution is determined by titration with a standard solution of an acid (usually hydrochloric acid), using *phenolphthalein* as indicator. The amount of potassium hydroxide

required for saponification is thus found by difference, and from this the saponification value calculated. It is obviously necessary to use phenolphthalein as indicator, as if methyl orange is used a red colour would only be produced when all the soap produced in the saponification had been decomposed, the acids set free from the soaps by the hydrochloric acid being without action on the methyl orange (see Vol. I., p. 65).

Weigh out from 2 to 2·5 gms. of olive oil (to the third place of decimals), placing the oil in a conical flask of about 250 c.c. capacity (see p. 33 for method of weighing out the oil). Add 25 c.c. of approximately semi-normal alcoholic potash solution,* measured with a pipette. Attach an upright (reflux) water condenser to the flask and heat on gauze over a small flame, so that the liquid boils gently for about half an hour.

The alcoholic potash should be standardised in a blank experiment. A solution of hydrochloric acid which is either exactly semi-normal or of which the factor is known is required for this purpose.

Owing to the possibility of absorption of carbon dioxide by the caustic potash during the saponification, it is more accurate to heat the alcoholic potash solution used in the blank experiment in the same manner and for the same length of time as in the test experiment.

Find the volume of standard hydrochloric acid solution required for 25 c.c. of the alcoholic potash solution after heating, using 10 drops of phenol phthalein as indicator.

When the saponification of the oil is complete, detach the condenser and cool the conical flask containing the saponified oil. Add 10 drops of phenol phthalein solution and titrate the excess of potassium hydroxide with the standard solution of hydrochloric acid, adding the acid until the pink colour is discharged. If the solution of alcoholic potash has been made up for some time it may be coloured, but there is usually no difficulty in deciding when the pink colour is discharged.

The saponification value is calculated from these results, as shown below.

From the equation

$$KOH + HCl \rightarrow KCl + H_2O$$

* This solution may be prepared by dissolving 8 gms. of caustic potash (purified by alcohol) in 10 c.c. of water, and adding 250 c.c. of "Industrial Methylated Spirit."

it will be seen that 56 gms. of potassium hydroxide are neutralised by 36·5 gms. of hydrogen chloride, or 1 litre of a normal solution of hydrochloric acid. Hence 1 litre of N/2 HCl is equivalent to 28 gms. of KOH, or 1 c.c. of N/2 HCl is equivalent to 28 milligrams of KOH. The volume of hydrochloric acid solution required for 25 c.c. of the alcoholic potash in the blank experiment, *less* the volume of the acid solution required in the test experiment, gives the volume of hydrochloric acid equivalent to the potassium hydroxide required for the saponification.

Example.—Weight of oil taken, 2·489 gms., 25 c.c. of alcoholic potash added, and the mixture boiled for half an hour. Volume of N/2 HCl for excess of KOH = 8·0 c.c.

In blank experiment—

25 c.c. of alcoholic potash required 25·4 c.c. N/2 HCl.

Volume of N/2 HCl equivalent to potash used for saponification = 25·4 − 8·0 = 17·4.

1 c.c. N/2 HCl = 28 mg. KOH.

∴ 17·4 c.c. N/2 HCl = 28 × 17·4 = 487·2 mg. KOH.

That is, 2·489 gms. of oil required 487·2 mg. KOH.

∴ 1 gm. of oil required $\frac{487{\cdot}2 \times 1}{2{\cdot}489}$ = 196 mg. KOH.

∴ Saponification value of olive oil used = 196.

The Saponification Values of Mixtures of Oils.

If a saponifiable oil such as olive oil were mixed with heavy petroleum oil such as "medicinal petroleum," which is, of course, unsaponifiable, the saponification value of the mixture is decreased in proportion to the amount of mineral oil present, and it is possible to calculate the extent of the adulteration from this value. Such mixtures are sometimes described as "salad oils." It should be noted that whilst olive oil is assimilated, petroleum oil passes through the body unchanged, and has therefore no food value.

If a mixture of olive oil (of saponification value 192) mixed with heavy petroleum gave a saponification value of 144, the percentages of the two oils in the mixture would be calculated as follows:—

192 mg. of KOH are required for 1 gm. of olive oil.

∴ 144 ,, ,, ,, $\frac{144}{192}$ = 0·75 ,, ,,

That is, 1 gm. of the mixture contained 0·75 gm. of olive oil and 0·25 gm. of petroleum; or, the oil contained 75 per cent. by weight of olive oil and 25 per cent. by weight of petroleum.

Determine the saponification value of a mixture of olive oil and medicinal petroleum, and calculate the percentages of the two oils present.

In carrying out the saponification of the mixture the flask should be shaken frequently to bring the olive oil into contact with the caustic potash.

If two saponifiable oils are mixed the calculation of the proportions in which they are present in the mixture would be made as follows:—

Saponification value of oil A = 250
" " " B = 190
" " mixture = 225

If 1 gm. of the mixture contains x gm. of A, then 1 gm. of the mixture contains $1 - x$ gm. of B.

1 gm. of A requires for saponification 250 mg. KOH.
(i) $\therefore x$ " " " " $250x$ " "
1 gm. of B requires for saponification 190 mg. KOH.
(ii) $\therefore 1 - x$ " " " $190(1 - x)$ " "

Adding (i) and (ii)—

$x + 1 - x$ gm. (= 1 gm.) of mixture requires for saponification $250x + 190 - 190x$ mg. KOH.

This by experiment in the example given = 225 mg. KOH.

$$\therefore 250x + 190 - 190x = 225.$$
$$60x = 35.$$
$$x = 0{\cdot}583.$$

That is, 1 gm. of the mixture contains 0·583 gm. of A and $1 - 0{\cdot}583 = 0{\cdot}417$ gm. of B.

Or, the oil consists of a mixture containing 58·3 per cent. by weight of A and 41·7 per cent. of B.

It should be noted that if an oil contains *free* acid some of the caustic potash required apparently for saponification will have been neutralised by this acid, and allowance must be made for this if the *true* saponification value (or *ester* value) is required (see "Acid Value," p. 46).

Unsaponifiable Matter in Oils and Fats.—Unadulterated saponifiable oils and fats contain usually less than 1 per cent.

of unsaponifiable matter, which consists largely of cholesterol or phytosterol (see p. 48).

The unsaponifiable matter is determined by saponifying a known weight of the material with alcoholic potash or soda and extraction of the unsaponifiable matter from the resulting soap solution by means of light petroleum. On evaporation of the light petroleum the unsaponifiable matter is obtained.

(See also Vol. I., p. 68, and "Fatty Foods," by Bolton and Revis.)

The Reichert-Meissl and Polenske Values of Oils and Fats.

Owing to the fact that glycerides as a rule are decomposed at temperatures below their boiling-points at atmospheric pressure, the constituents of a natural oil or fat cannot be separated by ordinary fractional distillation. A partial separation may, however, be obtained by vacuum distillation, but this is not usually carried out. A more important process is the partial separation of the acids obtained from the oils by hydrolysis. The mechanical separation of the liquid oleic acid from solid palmitic and stearic acid, with which it is usually mixed, is a process carried out on a large scale in connection with the manufacture of soap, candles, etc.

Owing to differences in solubility of the silver or magnesium salts of the acids, separation is possible by what is known as fractional precipitation. The fact that lead oleate is soluble in ether is sometimes made use of for the separation of oleic acid from a mixture. The most important method of examination of the acids obtained from fats depends, however, on differences in their degree of volatility when distilled with water (distillation in steam).

In this process the sodium salts of the acids obtained by saponification of the fats are treated with very dilute sulphuric acid, whereby the acids are set free. On distillation of the mixture, the acids of low molecular weight being volatile pass into the distillate with excess of water. Some of these acids are soluble in water, and others only very slightly soluble; so that if the distillate is filtered the acids volatile in steam and *soluble* in water may be determined by titration with a standard solution of an alkali, using phenolphthalein as indicator. If the distillation is carried out in an apparatus of certain specified dimensions, the volume of decinormal

sodium hydroxide required for the neutralisation of these acids, obtained from 5 grms. of a fat, is known as the *Reichert-Meissl value* of this fat. This determination is of great importance in connection with the analysis of butter and margarine (see p. 55). The acids which are volatile in steam and insoluble in water, which have been removed by filtration of the distillate, are dissolved in alcohol and their amount determined by titration. The volume of decinormal sodium hydroxide required for neutralisation of the acids which are volatile in steam and *insoluble* in water, obtained from 5 grms. of the fat, is known as the *Polenske value* of the fat. This determination may, of course, be carried out at the same time as the Reichert-Meissl process.

The chief difference in chemical composition between butter and margarine fats is that in the former tributyrin is present, and this on hydrolysis gives butyric acid, which is volatile in steam and soluble in water. Hence butter fat has a high Reichert-Meissl value, whereas animal fat margarine (oleomargarine), which does not contain glycerides derived from acids of low molecular weight, has a very low Reichert-Meissl value. The Reichert-Meissl and Polenske values of certain oils and fats are given below :—

	Reichert-Meissl Value.	Polenske Value.
Butter fat . . .	24-30	1·5-3
Oleomargarine fat . .	Less than 1	Less than 1
Coconut oil . . .	6-8	15-18

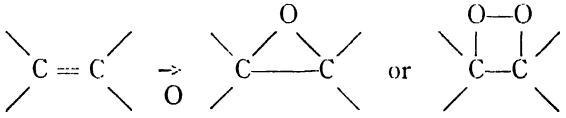
In addition to the high *Reichert-Meissl* value for butter previously noted, the high *Polenske* value for coconut oil should be noted. The latter value is of importance in relation to the analysis of vegetable fat margarines, and, if high, indicates that this fat contains considerable proportions of glycerides derived from acids which are volatile in steam and insoluble in water (see table, p. 31). Palm kernel oil resembles coconut oil in having a high *Polenske* value. The practical determination of the *Reichert-Meissl* and *Polenske* values is dealt with under butter and margarine, page 56. An important extension of the *Reichert-Meissl-Polenske* process is met with in connection with the determination of what is known as the *Kirschner* value. In this process a partial separation of the acids in the distillate obtained in the first

process is effected by means of their silver salts. This value is of use in determining the amount of butter fat in margarine.

The Iodine Value of an Oil or Fat.

Drying and Non-Drying Oils.

One of the most important considerations in connection with an oil is whether or not it will harden on exposure to air. Thus it is essential, for example, that a lubricating oil should not dry on exposure to air; but, on the other hand, it is necessary that an oil used in the manufacture of paint should harden when so exposed. Amongst the saponifiable oils, in general, those derived from *unsaturated* acids will dry on exposure to air owing to absorption of oxygen, this absorption of oxygen taking place at the point of unsaturation of the molecule, a solid substance being produced.



Thus the linseed oil in paint does not dry owing to evaporation but to absorption of oxygen (see Vol. I., p. 267).

As is well known, unsaturated substances form compounds with halogens by direct addition, and this reaction between an oil, containing an unsaturated glyceride, and iodine is employed in the determination of what is known as the *iodine value* of an oil. This represents the percentage proportion of iodine absorbed by the oil, or the number of grams of iodine absorbed by 100 gms. of the oil. In general, the higher the iodine value of an oil the more readily will it dry on exposure to air.

In the original process for the determination of the iodine value of an oil, known as Hübl's process, the oil was treated with an alcoholic solution of iodine containing mercuric chloride. In the Wijs modification of the process iodine monochloride, ICl, is employed instead of free iodine, as the time required for complete saturation with this reagent is considerably less than with free iodine. The reaction is represented as follows :—

$$-CH=CH- + ICl \rightarrow -CHI-CHCl-$$

In the actual determination a weighed quantity of the oil, dissolved in carbon tetrachloride, is treated with a measured volume of iodine monochloride solution. The mixture is allowed to stand for a certain time, and the excess of iodine monochloride then estimated, after the addition of potassium iodide, by means of a standard solution of sodium thiosulphate. The reactions which take place are as follows :—

$$ICl + KI \rightarrow KCl + I_2$$
$$2Na_2S_2O_3 + I_2 \rightarrow 2NaI + Na_2S_4O_6.$$

It is essential that at least half the iodine monochloride originally present should remain in the solution after the absorption by the oil has taken place. This ensures that excess of the reagent is in contact with the oil throughout the absorption. On this account only a very small quantity of a drying oil should be used for the experiment.

From the difference in volume of thiosulphate solution required for a certain volume of iodine monochloride solution, in a blank experiment, and that required for the same volume of iodine monochloride solution after treatment with the oil, the amount of iodine absorbed by the given weight of oil is found, and hence its iodine value may be calculated. In reality, as pointed out above, it is iodine monochloride and not iodine which is absorbed by the oil; but this does not affect the amount of thiosulphate required in either the blank or test experiment, since one molecule of iodine monochloride with potassium iodide gives one molecule of iodine.

The various solutions required for the determination of the iodine value of an oil are prepared as follows :—

Decinormal sodium thiosulphate solution, 24·8 gms. of the crystallised salt $Na_2S_2O_3 . 5H_2O$ per litre (see Vol. I., p. 137).

Starch solution prepared as described in Vol. I., p. 16.

Potassium iodide solution.—A solution containing 10 gms. of the salt per 100 c.c. of water.

The Wijs iodine monochloride solution.—3·2 gms. of finely powdered iodine are dissolved in acetic acid (98-99 per cent.) and the solution diluted with the same solvent to 250 c.c. in a graduated flask.

5 c.c. of the solution are treated with about 3 c.c. of 10 per cent. potassium iodide solution and 30 c.c. of water. The decinormal sodium thiosulphate solution is then run in

from a burette until the colour of the iodine is practically removed. A few drops of starch solution are added, and the sodium thiosulphate run in drop by drop until the blue colour is discharged. The amount of sodium thiosulphate solution required is noted.

Pure dry chlorine is then passed into the remainder of the iodine solution until the colour of the solution is changed from brown to red, and until 5 c.c. of the solution, treated as before, requires twice as much sodium thiosulphate to remove the iodine as when first prepared.

The pure dry chlorine is prepared by dropping concentrated commercial hydrochloric acid on to solid potassium permanganate, the gas being passed through wash bottles containing (*a*) water to remove hydrochloric acid; (*b*) sulphuric acid to remove aqueous vapour, before being passed into the iodine solution.

The iodine monochloride solution may, if preferred, be made up by dissolving a weighed quantity of iodine trichloride in glacial acetic acid and adding sufficient iodine to convert the trichloride into monochloride.

In a determination of the iodine value of an oil the following results were obtained :—

Weight of beaker + rod + oil	=	29·685 gms.
,, ,, ,, + part of oil	=	29·525 ,,
Weight of oil taken		0·160 ,,

This weighed quantity of oil, which was placed in a dry glass-stoppered bottle of about 300 c.c. capacity, was treated with 10 c.c. of carbon tetrachloride and 25 c.c. of the iodine monochloride solution. (The carbon tetrachloride is used to act as a solvent for the oil, and so bring it all into contact with the iodine monochloride.) The bottle was then tightly stoppered, the stopper having been previously moistened with potassium iodide solution to prevent loss of iodine, and the mixture allowed to stand in the dark for one hour.

*Blank Experiment.**—25 c.c. iodine monochloride + 10 c.c.

* Owing to the fact that the carbon tetrachloride may contain some substance which will absorb iodine, it is better to allow the mixture of this substance and the iodine monochloride to stand for an hour, as in the test, before addition of the potassium iodide and titration.

CCl_4 + 15 c.c. 10 per cent. KI solution + 150 c.c. water + a few drops of starch solution, required for removal of the iodine—

(i) 49·6 c.c. N/10 $Na_2S_2O_3$
(ii) 49·6 c.c. ,, ,,

(*Note.*—The starch is not added until the colour of the iodine is practically discharged.)

Test Experiment.—The iodine monochloride solution which has been in contact with the oil was then treated with potassium iodide solution, water and starch solution as in the blank experiment, and sodium thiosulphate solution run in.

Volume of N/10 thiosulphate required = 26·8 c.c.

Amount of iodine monochloride absorbed by the oil is thus equivalent to

$$49{\cdot}6 - 26{\cdot}8 = 22{\cdot}8 \text{ c.c. N/10 } Na_2S_2O_3,$$

but 1 c.c. N/10 $Na_2S_2O_3 = 0{\cdot}0127$ gm. iodine.

$\therefore$ 22·8 c.c. ,, ,, $= 0{\cdot}0127 \times 22{\cdot}8 = 0{\cdot}2896$ gm. iodine.

This iodine is equivalent to the iodine monochloride absorbed by the oil.

$\therefore$ 0·16 gm. of the oil would absorb 0·2896 gm. iodine, or 100 gms. would absorb $\frac{0{\cdot}2896}{0{\cdot}16} \times 100 = 181$ gms. iodine, or iodine value of the oil = 181.

The iodine values for various oils are given in the table below. Those which have iodine values above 120 are usually called *drying* oils, those of which the iodine value is between 95 and 120 *semi-drying* oils, and those having an iodine value less than 95, are known as *non-drying* oils.

Oil.	Iodine Value.
Arachis	87-98
Castor	85-95
Coconut	8-10
Cottonseed	104-110
Linseed	175-200
Olive	80-86
Palm	52-57
Rape	95-100
Whale	110-146
Cod liver oil	160-180

Hydrogenation of Oils and Fats.

As a general rule glycerides of unsaturated acids have comparatively low melting-points and often unpleasant odours; that is, oils and fats having high iodine values are often of a softer consistency than those having low iodine values. Fish oils as a rule have high iodine values, and are low melting-point substances; on the other hand, a fat containing a large amount of saturated glycerides derived from acids of high molecular weight, such as mutton fat, has a high melting-point.

The hardening and deodorising of oils by the conversion of unsaturated into saturated glycerides by the addition of hydrogen forms the basis of a very important technical process known as *hydrogenation*.

This addition of hydrogen takes place when a mixture containing unsaturated glycerides is heated with hydrogen in the presence of very finely divided nickel or other catalyser, the oil being thereby hardened and deodorised. Thus it is sometimes possible to use oils, which have been subjected to this process, for the manufacture of margarine; although, owing to their low melting-points or objectionable odours, these oils are quite unfit for use for this purpose before hydrogenation. The reaction involved in this process is represented in the case of triolein by the equation given below, the product being tristearin:—

$$\begin{array}{l} CH_2OOC.C_{17}H_{33} \\ | \\ CHOOC.C_{17}H_{33} \\ | \\ CH_2OOC.C_{17}H_{33} \end{array} + 6H \rightarrow \begin{array}{l} CH_2OOC.C_{17}H_{35} \\ | \\ CHOOC.C_{17}H_{35} \\ | \\ CH_2OOC.C_{17}H_{35} \end{array}$$

Triolein. Tristearin.

Thus derivatives of oleic acid, $C_{17}H_{33}COOH$, are converted into derivatives of stearic acid, $C_{17}H_{35}COOH$, by the addition of two atoms of hydrogen for each oleic acid radical. Similarly, derivatives of linolic acid, $C_{17}H_{31}COOH$, and linolenic acid, $C_{17}H_{29}COOH$, are also converted into derivatives of stearic acid.

The equation for the addition of iodine to triolein would differ only in substituting six atoms of iodine for the six atoms of hydrogen, the product being $C_3H_5(OOCC_{17}H_{33}I_2)_3$,

that is, tristearin in which six atoms of hydrogen are replaced by six atoms of iodine.

It will be obvious that if an oil has been completely hydrogenated, its iodine value is reduced to zero, but it should be noted that its saponification value is not affected to any great extent. Thus the iodine value of triolein, $C_3H_5(OOCC_{17}H_{33})_3$, is 86·2 and its saponification value 190, the corresponding values for tristearin, $C_3H_5(OOCC_{17}H_{35})$, being 0 and 188·8.

A laboratory method for the hydrogenation of volatile substances such as benzene, C_6H_6, and toluene, $C_6H_5 . CH_3$, which are converted into hexahydrobenzene (cyclohexane), C_6H_{12}, and hexahydrotoluene, $C_6H_{11} . CH_3$, is described in "Chemistry of Petroleum."

The technical processes dealing with hydrogen are described in "The Hydrogenation of Oils," by Ellis (Constable).

Detection of Hydrogenated Oils.—The usual method is to test for the presence of a minute amount of nickel by means of a colour reaction with a benzil dioxime,

$$C_6H_5C(NOH)C(NOH)C_6H_5$$

(see Allen, "Commercial Organic Analysis," Vol. IX.). The absence of nickel cannot be taken as a proof of the absence of a hydrogenated oil, as either the nickel may have been completely removed, or some other catalyser may have been employed.

With a view to the estimation of the amount of unsaturated glyceride present, in addition to the determination of the iodine value of an oil, use is made of the fact that heat is evolved when bromine is absorbed by an unsaturated glyceride, the larger the percentage of unsaturated glyceride present, the greater the rise in temperature noted when the oil is treated with bromine. This affords another method of distinguishing between oils. For further details of the method, see "Food Inspection and Analysis," by Leach.

The Acid Value of an Oil or Fat.

Naturally occurring mixtures of glycerides usually contain a *small* amount of free fatty acid produced by hydrolysis. This proportion of acid increases slightly if the fat becomes rancid (see p. 49). The amount of this free acid in a mixture of glycerides is determined by titration of a solution of the

glyceride, in a mixture of alcohol and ether, with a standard solution of potassium or sodium hydroxide, using phenolphthalein as indicator. The *acid value* of a fat is the number of milligrams of potassium hydroxide required to neutralise the free acids in 1 gm. of the fat.

The presence of free fatty acid in saponifiable oils appears to be of great importance in connection with their use as lubricants. Mixtures of heavy petroleum oils and saponifiable oils are often used for this purpose, and it has recently been found that a small amount of the fatty acids of a saponifiable oil can replace a large amount of the saponifiable oil in a lubricant.

For experience of the determination, the acid value of beeswax should be determined, although of course this substance is not an edible oil or fat.*

Determination of the Acid Value of Beeswax.—Weigh out accurately from 3 to 4 gms. of shredded beeswax, place in a conical flask, and add about 20 c.c. of 96 per cent. alcohol. Warm the mixture until the wax is melted, and titrate with a seminormal solution of alcoholic potash, using phenolphthalein as indicator. Keep the mixture warm during the titration, and shake well after each addition of alkali.

From the volume of alcoholic potash required calculate the acid value of the beeswax. For most samples of the substance this is approximately 20.

The approximate amount of beeswax in a mixture with paraffin wax † may be calculated from the result obtained for the acid value of the mixture, assuming the acid value for beeswax, as described under saponification value of a mixture of oils, p. 37.

For the more complete analysis of such a mixture the saponification value should be determined. In the case of beeswax, however, it is necessary to boil the wax with alcoholic potash for about an hour to ensure complete saponification.

* The term wax is usually applied to substances which when distributed over a surface by means of a solvent are capable of taking a polish. Paraffin wax, a mixture of solid hydrocarbons, is quite different in composition from most waxes which are essentially esters or mixtures of esters and free acids. These esters are derived from monhydric alcohols, and are thus not glycerides.

Beeswax, for example, is a mixture of the melissyl ester of palmitic acid, $C_{15}H_{31}COOC_{30}H_{61}$ (derived from melissyl alcohol, $C_{30}H_{61}OH$, and palmitic acid, $C_{15}H_{31}COOH$), and free cerotic acid, $C_{25}H_{51}COOH$.

Chinese wax consists chiefly of ceryl cerotate, $C_{25}H_{51}COOC_{26}H_{53}$.

Spermaceti consists chiefly of cetyl palmitate, $C_{15}H_{31}COOC_{16}H_{33}$.

† See "Furniture Polishes," Vol I., p. 274.

The saponification value of beeswax varies from 92 to 97, being usually approximately 95. Deducting 20 for the acid value, the *true* saponification or *ester* value is 75. The ratio 20 : 75, or 1 : 3·75 is practically constant for all genuine samples of beeswax and is of use in detecting certain adulterants in samples of the wax.

Other Constants, etc., of Oils.

In addition to the determination of the constants previously dealt with, many other tests are carried out in the complete examination of an oil. These, however, will only be dealt with very briefly.

The Acetyl Value of an Oil.—A derivative of a hydroxy acid, such as ricinoleic acid, on heating with acetic anhydride is acetylated, that is, the hydrogen atoms of the hydroxyl groups in the radicals are replaced by acetyl groups. These esters of acetic acid which are formed will give acetic acid on hydrolysis, the amount produced depending on the amount of hydroxy acid derivative present in the original mixture. Thus different results are obtained with different oils.

The reactions involved are as follows :—

$$\underset{\text{Derivative of ricinoleic acid.}}{C_{17}H_{32}(OH)COOX} + \underset{\text{Acetic anhydride.}}{(CH_3CO)_2O} \rightarrow \underset{\text{Acetylated substance.}}{C_{17}H_{32}(OOCCH_3)COOX} + \underset{\text{Acetic acid.}}{CH_3COOH}$$

$$C_{17}H_{32}(OOCCH_3)COOX \xrightarrow[\text{Hydrolysis.}]{} CH_3COOH + C_{17}H_{32}(OH)COOH.$$

The acetic acid produced by hydrolysis is separated from acids of high molecular weight as in the *Reichert-Meissl* determination. The number of milligrams of caustic potash required for the neutralisation of the acetic acid obtained by the hydrolysis of 1 gm. of the acetylated fat is known as the *acetyl* value of that fat.

In carrying out the process a correction would be necessary for the acids which are volatile in steam and soluble in water, obtained by hydrolysis of the original fat.

The Valenta Test.—Oils differ considerably in their solubility in glacial acetic acid. In this test the *temperature* is noted at which the oil separates from solution on cooling.

Cholesterol and Phytosterol in Oils and Fats.—These isomeric monohydric alcohols are found in very small amounts as unsaponifiable substances in animal and vegetable fats

respectively. In virtue of their alcoholic nature they form acetates, the melting-point of cholesteryl acetate being 113° C., and of phytosteryl acetate 125° to 137°, according to the source of the phytosterol, different vegetable oils containing different phytosterols. The separation of these alcohols and the preparation and purification of their acetates is an important operation in connection with the detection of a vegetable fat in an animal fat. Thus if the melting-point of the acetate is above 116° C., the presence of phytosterol, and hence a vegetable fat in the original mixture, is indicated.

In order, however, to mask the adulteration of an animal fat by the addition of a vegetable fat, a very small amount of paraffin wax (of which the m.p. is considerably below 100° C.) is sometimes added with the vegetable substance, so as, to reduce the melting-point of the phytosteryl acetate to that of cholesteryl acetate.

This addition is sometimes met with in the adulteration of lard by the addition of vegetable oils.

For further information on this subject, see "Fatty Foods," by Bolton and Revis.

Rancidity.—Comparatively little is known with certainty concerning the chemical changes which take place when a fat becomes rancid. The unpleasant odour and taste of rancid fat may become distinct without any appreciable change in any of the physical and chemical constants of the substance being noted. There is, however, apparantly no doubt that in the case of unrefined oils pronounced rancidity leads to increased free fatty acid content, the glycerides being hydrolysed in the presence of moisture by a fat-splitting enzyme. The products of hydrolysis, glycerol and fatty acid, may then be oxidised, but it is not known whether the objectionable substances in rancid fat, which are probably present in very small amounts, are oxidation products of the glycerol or fatty acid.

It is well known that rancidity develops more rapidly if the moist oil is exposed to air and light, and in some cases it appears to be initiated by the growth of moulds on wooden containers for the fat. For example, if butter fat is carefully freed from water it may be stored for a considerable time without becoming rancid. It is usually so obvious from its taste and smell when a fat has become rancid that no chemical test for rancidity is needed, although certain colour reactions are given by rancid fats.

The removal of the objectionable products from a rancid fat is usually effected by blowing a current of air through it whilst in the molten condition. Thus in the production of "renovated" or "process" butter, a product sold under certain regulations in various parts of America, the fat separated from rancid butter is treated with a current of air and then churned with milk.

BUTTER AND MARGARINE.

Butter consists of a more or less homogeneous mixture of milk fat with water and small quantities of the other constituents of milk, such as protein, usually referred to as curd; together with traces of lactose and mineral salts. In addition it contains as a rule added common salt, and in many cases boric acid or borax as a preservative. The maximum amount of water allowed under the "Sale of Butter Regulations" is 16 per cent. Boric acid or borax may be present to the extent of 0·5 per cent., but its use may shortly be prohibited.

In margarine the milk fat is replaced either by a vegetable fat such as coconut, palm kernel, or arachis oil; or by an oil or fat of animal origin, such as oleo oil prepared from beef fat. Margarine made entirely from animal fat is often referred to as "oleomargarine." In some varieties of margarine both animal and vegetable oils are used.

The chief difference from a chemical point of view between butter and margarine is thus in the nature of the glycerides of which the fat is composed (see p. 31).

Another difference which may be of importance is that the accessory factor known as vitamin A present in butter fat may possibly be absent from margarine made entirely from vegetable fat. (See "The Analyst," 1922, **47**, 240, and the papers therein referred to.)

The Manufacture of Margarine.*

Preparation of the Fats.

(*a*) *Animal Fats.*—The fresh animal fats are chilled and cut into small pieces, after which the temperature is raised to about 150° F., and the clear oil known as "premier jus"

* For full details of the processes employed for this purpose, see "Margarine," by Clayton (Longmans).

drawn off and allowed to stand. The solid fat which separates, known as "oleo stearine," is separated mechanically from the liquid portion known as "oleo oil." This liquid portion, which is used for the manufacture of margarine, contains a certain amount of triolein. By careful regulation of the temperature at which the separation is effected, and of certain mechanical details, the proportions of glycerides of low or high melting-points in the oleo oil can be controlled. Thus, if the fat is required for use in the summer, a larger amount of glycerides of high melting-point will be left in at this stage than will be the case in winter, when a lower melting-point fat is required for the margarine. The oleo oil sets at the ordinary temperature to a pale yellow fat.

(*b*) *Vegetable Oils and Fats.*—The raw materials copra (dried coconut), cottonseed, palm kernels, arachis pods, etc., are imported, the oils being extracted from them in this country. The crude oils, after separation from the oleaginous material by pressure, are washed with hot water, the suspended matter is removed, and they are then bleached by filtration through animal charcoal or various silicates. Free fatty acid in the oil is then neutralised, and the oil is treated with superheated steam to remove volatile substances which have an unpleasant odour.

The oils which are obtained are practically without colour, taste, or smell. Coconut and palm kernel oils are solid at temperatures below 24° and 28° C. respectively, and owing to the fact that in a works it is easier to transport material in the liquid than the solid state, these oils are often delivered in the liquid state in insulated tanks at a temperature of about 40° C.

In addition to the oils mentioned above, hydrogenated oils (see p. 45) are used in the manufacture of certain brands of margarine.

Skimmed Milk for use in Margarine Manufacture.

This material serves a double purpose in connection with the manufacture of margarine. It is useful in making an emulsion of the fat and, by the use of soured skimmed milk, a flavour resembling, to a certain extent, that of butter is imparted to the product. In a margarine works the whole milk is usually first gently heated to facilitate the removal of cream, which is effected by means of a centrifuge. The

cream obtained is not used for the manufacture of margarine, but is usually made into butter. The skimmed milk is then sterilised, cooled, and soured by the addition of a pure culture of a lactic acid forming bacillus. This operation is one of great importance, as the introduction of other bacilli may spoil the flavour of the finished product. The progress of the souring process may be followed by titration (see p. 19), and when this has proceeded far enough the milk is cooled to check the lactic fermentation, and the soured skimmed milk thus obtained is ready for use.

Emulsification, etc.

A mixture of oil, soured skimmed milk and colouring matter is introduced into machines known as emulsifiers. The oil is broken up into very fine particles by means of paddles, which rotate at a very high velocity. For the production of a satisfactory product efficient emulsification is absolutely necessary, the aim being to reduce the size of the globules of oil to as nearly as possible that of the particles in butter fat. The colouring matter which is added is often an azo dye of the nature of methyl orange.

The liquid emulsion, at a temperature of about 30° C., is allowed to fall from a perforated pipe between two large cylinders mounted horizontally and placed so that their sides are nearly in contact. The cylinders revolve in opposite directions. The perforated pipe is placed parallel to the cylinders, and the perforations extend over a length of it slightly less than the length of the cylinders. These cylinders are filled with brine, which circulates through them at a temperature well below zero (see p. 204). The emulsion falling on the cylinders is thereby cooled *suddenly*, and it solidifies immediately. The solidified product is removed from the cylinders by means of knife edges, and is collected in a truck below.

The sudden cooling of the emulsion prevents the formation of large crystals, which would give the margarine a coarse-grained consistency, which is unsuitable. The solidified emulsion is then usually kept in a store-room at about 17° C. The lactic acid forming bacilli again become active and the flavour of the product is improved. The mixture is then churned to improve its texture, after which salt and preservative (usually a mixture of boric acid and borax to the extent,

of about 0·5 per cent.) are added.* Since the final product must not contain more than 16 per cent. of water, it is important, from the manufacturer's point of view, to make sure that at this stage a portion of the product, which may contain more than this amount of water, is blended with a portion containing less than this amount.

The product is now ready for sale, and is packed in boxes which must be marked "Margarine" in letters of a certain size. Definite regulations as to marking of the wrappers when it is sold retail have also to be complied with.

In some cases butter is mixed with margarine by the manufacturer,* but the amount of butter fat must not exceed 10 per cent. by weight, and the mixture must be labelled "Margarine."

The Analysis of Butter and Margarine.

The operations with which we are concerned in this connection deal with determinations of the percentages of water, fat, curd, and salt; the examination of the fat by the *Reichert-Meissl* and *Polenske* methods, and the detection of preservative and added colouring matter.

Determination of Water, Fat, Curd and Salt in Butter or Margarine.

For the determination of water, a weighed amount of the substance is heated at 100° C. until a constant weight is obtained; the fat is removed by solution in light petroleum, and its amount calculated from the loss in weight. The curd and salt remaining are then ignited, the loss in weight representing the curd, and the residue gives the weight of mineral matter (largely common salt) in the material taken for analysis. These constituents may be determined with a fair degree of accuracy as follows:—

Water.—A silica Gooch crucible is half filled with purified teased asbestos, which is lightly pressed down. The crucible is then heated first by means of a small Bunsen flame, and finally for about ten minutes in a muffle furnace. It is then allowed to cool in a desiccator, and is weighed when quite cold.

* In some cases the salt and preservative are added to the mixture before emulsification. It is probable that the addition of any preservative to butter or margarine will shortly be illegal.

About 2 gms. of butter or margarine, representative of the whole bulk of the material under examination, are then introduced, and the crucible and contents again weighed. The crucible is now placed in a steam oven at 100° C., where it is allowed to remain for about an hour, after which it is cooled in a desiccator and weighed. It is then replaced in the oven and heated for half an hour, and again cooled and weighed. The process is repeated until the weight is constant.

From the loss in weight the percentage of water in the material is calculated.

Fat.—The crucible is placed in a *small* beaker and light petroleum poured into the beaker and crucible to the same height, and until the asbestos is just below the surface of the liquid. The beaker is covered to prevent evaporation of the light petroleum, and the crucible is allowed to remain in the solvent for about a quarter of an hour, when it is transferred to its funnel attached to a filter flask. The solution of fat in the crucible is then removed by *gentle* suction, and the crucible again transferred to the beaker and its contents treated with light petroleum as before.*

After ten minutes the crucible is again transferred to its funnel in the filter flask and the liquid removed as before. The contents of the crucible are then washed with successive small quantities of light petroleum, using gentle suction, until a drop of the liquid coming through the funnel leaves no residue of fat when evaporated to dryness on filter paper. The crucible is dried in the steam oven, cooled, and weighed. The loss of weight, when a constant weight has been obtained, due to the extraction of the fat, gives the weight of fat in the amount of material taken, and from this the percentage of fat is calculated.

Curd.—The crucible is now gently heated over a Bunsen flame in the draught cupboard to remove organic matter (protein), and, finally, for ten minutes in a muffle furnace.

The loss of weight produced by this operation gives the amount of "curd" in the material taken for the experiment, from which the percentage of this constituent is calculated in the ordinary way. It should be noted that this loss of weight on ignition is partly due to the removal of traces of lactose, etc., as well as protein.

* The light petroleum extracts containing the fat in solution should be kept, and may be freed from fat by distillation later.

Salt.—The weight of the crucible and contents after ignition, less the original weight of the crucible and asbestos, gives the amount of the "salt" in the weight of substance taken, and hence the percentage of this constituent may be calculated.

The ash obtained in this experiment, although very largely composed of common salt, contains, of course, all the mineral matter originally present in the portion of substance taken for analysis. If the percentage of salt alone is required, this constituent may be removed from the ash by means of hot water, the solution diluted to a known volume, and the amount of sodium chloride in a portion of the solution determined by titration with decinormal silver nitrate solution, using potassium chromate as indicator (see Vol. I., p. 9).

1 c.c. $N/10 AgNO_3 = 0{\cdot}00585$ gm. NaCl.

The results obtained in the analysis of butter and margarine usually lie between the limits given below:—

Water	9	to 15	per cent.
Fat	80	,, 90	,,
Curd	0·5	,, 1·5	,,
Ash (including salt)	0·5	,, 5	,,

The Examination of Butter and Margarine Fat.

In this connection we shall deal only with the determination of the *Reichert-Meissl* and *Polenske* values, although it must be pointed out that many other determinations are usually necessary in order to be able to give any opinion of value, for example, as to whether butter has been adulterated by the addition of even a comparatively large amount of a foreign fat.

The principles involved in the determination of the *Reichert-Meissl* and *Polenske values* have been dealt with previously (see p. 39). Unadulterated butter fat gives a *Reichert-Meissl value* which is usually greater than 24, and may be over 30, the former value being taken as a minimum for genuine butter. If, therefore, a fat such as lard, of low *Reichert-Meissl value*, is added to a butter with a high value for this constant, a considerable quantity of the fat might be added and the *Reichert-Meissl value* of the mixed fat still be greater than 24. If, however, the adulterant is a substance

such as coconut oil, which itself has an appreciable *Reichert-Meissl value* (see p. 40), a still larger amount could be added without reducing the *Reichert-Meissl value* below 24. In the latter case, however, a determination of the *Polenske value* usually affords evidence of the addition of such a substance, as this value is high for coconut oil and low for butter. An animal fat such as lard, however, has a very low *Polenske value*, and the addition of this substance, which had not been detected by a determination of the *Reichert-Meissl value*, would still be undetected when the *Polenske value* had been determined.

It will be seen therefore that the question of the detection of adulteration of butter is a very complex one. The subject is dealt with fully in "Fatty Foods," by Bolton and Revis, and for further information on the subject that book should be consulted.

Determination of the Reichert-Meissl and Polenske Values.

Separation of the Fat from Butter and Margarine.—The butter or margarine is placed in a beaker, or evaporating basin, in a hot water oven and kept at about 60° to 70° C., until the water and curd have separated from the fat. The clear fat is then poured off from the water and curd through a dry fluted filter paper in a funnel, fitted with a hot water jacket (see p. 33), to prevent solidification in the stem of the funnel. The fat is collected in a small beaker, any drops of water which have passed through the funnel being removed from the fat in the beaker by means of strips of filter paper.

Saponification.—Weigh accurately as nearly as possible 5 gms. of the fat into a 300 c.c. flat-bottomed flask.

To do this, place in a dry porcelain dish approximately 5·1 gms. of the melted fat, allow to cool, and weigh accurately the dish containing the fat. Warm the dish *slightly*, and pour the melted fat into the flask. Weigh the dish again when cold, and the difference in weight will give the weight of fat taken.

To the contents of the flask add 20 gms. of glycerol, and then by means of a graduated pipette add 2 c.c. of a concentrated solution of sodium hydroxide * (made by dissolving 100 gms. of sodium hydroxide in 100 c.c. of water).

* It is advisable to use a water pump for drawing this solution into the graduated pipette.

Heat the flask over a gauze, shaking the contents frequently, until a clear solution is obtained. About ten minutes' heating will be required.

By this process the fat is converted into soaps.

Distillation with Dilute Sulphuric Acid.—Allow the mixture in the flask to cool slightly, and then add gradually 100 c.c. of recently boiled distilled water. When the soap is dissolved add about 0·2 gm. of very finely powdered pumice. This is to prevent bumping in the subsequent distillation, owing to superheating of the liquid below the oily layer of fatty acid. Now add 50 c.c. of a solution of sulphuric acid, which contains 28 c.c of concentrated sulphuric acid in 1 litre. Attach the distillation tube and condenser, which in accurate work should be of the dimensions shown in the diagram in mm. (Fig. 8). The distillate is to be collected in a 110 c.c. graduated flask, which is *not*, of course, fitted to the condenser by means of a cork. Heat the flask at first over a small flame on a piece of wire gauze until the acids which have separated out are melted; then heat more strongly and at such a rate that 110 c.c. of distillate will be collected in approximately half an hour. When this volume of distillate has been collected, stop the distillation and cool the distillate, by allowing the flask to stand in cold water (at 10° to 15° C.) for about ten minutes.

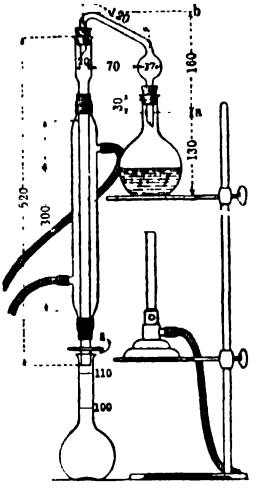


FIG. 8.—Reichert-Meissl-Polenske Apparatus.

Note the appearance of the insoluble acids floating on the distillate, as, if the fat under examination contains an appreciable quantity of coconut oil, liquid acids will separate in the form of oily drops.

Titration of Acids Volatile in Steam and Soluble in Water.—Filter the distillate, after mixing by shaking, through a dry filter paper,* and titrate 100 c.c. of it with decinormal sodium hydroxide solution, using *phenolphthalein* as indicator. The volume of sodium hydroxide solution required multiplied by

* Keep the filter paper employed in this experiment for use later (*Polenske value*).

1·1 (as 100 c.c. only of the 110 c.c. of distillate was titrated) gives the volume of decinormal sodium hydroxide solution required for the acids volatile in steam and soluble in water. Calculate the volume which would have been required if exactly 5 gms. of fat had been taken. This gives the *Reichert-Meissl* value of the fat.

Titration of Acids Volatile in Steam and Insoluble in Water.—Pour 18 c.c. of distilled water through the condenser used in the experiment into the 110 c.c. flask used for the distillate, and from this flask, after repeated shaking, over the filter paper through which the distillate was filtered. Reject the filtrate obtained, as this treatment is to free the insoluble acids from soluble acids. (This volume of water is the amount used in the standard method for carrying out the determination; if a large quantity of water were used, part of the so-called "insoluble" acids would be dissolved.)

Now dissolve the solid acids remaining in the condenser, 110 c.c. flask, and on the filter paper by washing with small quantities of 95 per cent. alcohol, which is neutral to phenolphthalein, or made neutral if necessary, using in all about 50 c.c. of alcohol.

Titrate the alcoholic solution of the acids with N/10 sodium hydroxide, using phenolphthalein as indicator. The volume of sodium hydroxide solution required, corrected for exactly 5 gms. of the fat, gives the *Polenske value* of that fat.

Interpretation of Results.

(*a*) *The Adulteration of Butter by the Addition of Animal Fats.*—Unadulterated butter fat should give a *Reichert-Meissl value* of not less than 24. In the case of the addition of animal fats, such as oleo oil or lard, which have very low *Reichert-Meissl* and *Polenske values*, the former value for the mixed fat may be less than 24 (see p. 55), and the latter will probably be less than 2. If the *Reichert-Meissl value* of such a mixture is assumed to be due entirely to the butter fat, the percentage of foreign fat in the butter is calculated as follows:—

If a value of 17·3 is obtained for the *Reichert-Meissl value*: then since a value of 24 for this constant corresponds to 5 gms. of unadulterated butter fat (see p. 55), a value of 17·3 for this constant corresponds to $\frac{5 \times 17{\cdot}3}{24}$ = 3·6 gms. butter fat.

Or 5 gms. of the mixed fat contains 3·6 gms. butter fat.

$$\therefore \text{ 100 } \text{,, } \text{ ,, } \text{ ,, } \frac{3{\cdot}6 \times 100}{5}$$

$$= 72 \text{ per cent. of butter fat.}$$

That is, the *fat* contains 100 — 72 = 28 per cent. of animal fat other than butter fat.

If the total percentage of fat in the sample is 80, then the percentage of added fat in the original sample of the material is calculated as follows :—

100 parts of *fat* in sample contain 28 parts of foreign fat.

$$\therefore \text{ 80 parts of } fat \text{ in sample} = 100 \text{ parts of sample of butter} \left\{ \text{contain } \frac{28 \times 80}{100} \text{ ,, ,,} \right.$$

$$= 22 \text{ parts}$$

Or the percentage of foreign fat in the sample of butter is 22.

(*b*) *The Adulteration of Butter by the Addition of a Vegetable Fat such as Coconut Oil.*—In such a case the *Reichert-Meissl value* may still be greater than 24, but the *Polenske value* will probably be greater than that of genuine butter fat. In the table given below the maximum *Polenske values* are given for various genuine butter fats having different *Reichert-Meissl values* :—

Reichert-Meissl Value.	Polenske Value should not exceed
24	2·2
26	2·5
28	3·2
30	3·5
32	4·0

Thus, if a butter fat of *Reichert-Meissl value* 28 gave a *Polenske value* greater than 3·2, coconut or palm kernel oil is probably present, but from the results of the experiments described here it is not possible to determine accurately the amount which has been added.

Another problem, closely related to the question of the adulteration of butter, is the determination of the percentage of butter fat in margarine, since the maximum amount allowed is 10 per cent. This problem is, however, a very complex one, and for further information reference should be made to "Fatty Foods," by Bolton and Revis.

Tests for Distinguishing between Butter and Margarine.

The most important test has been dealt with in connection with determination of the *Reichert-Meissl* and *Polenske values.*

Although it is often easy to distinguish between the two materials by taste, appearance, etc., in the case of margarines containing highly flavoured butter this is not so easily done.

A considerable difference is often noted between the behaviour of butter and margarine when heated. If 2 or 3 gms. of the material are heated in a spoon over a free flame, butter will usually boil quietly with the production of a considerable amount of foam, whereas margarine and "renovated" or "process" butter boil with much crackling and produce little foam.

In another test some of the fat is heated with milk and then allowed to cool in ice, the mixture being stirred with a wooden rod. In the case of butter fat a mixture which is more or less homogeneous is obtained, but with certain margarines the fat separates in large lumps.

Added Colouring Matter in Butter and Margarine.

The colouring matter usually employed in the manufacture of margarine is an azo dye. Annatto is also occasionally used for colouring both butter and margarine (see also p. 145). To test for the presence of an azo dye, heat some of the fat in a test tube with an equal volume of a mixture of concentrated sulphuric acid and glacial acetic acid (consisting of one part by volume of sulphuric acid and three of acetic acid). A pink colour in the acid solution, which separates on cooling, shows the presence of an azo dye.

Annatto may be tested for by shaking some of the fat with warm, dilute sodium hydroxide solution and testing the liquid as described under milk, p. 20.

Preservatives in Butter and Margarine.*

A mixture of boric acid and borax is the preservative most usually employed. This is present in practically all margarine, and its presence up to the extent of 0·5 per cent., calculated as boric acid, is not, as yet, regarded as an adulteration. Boron preservatives are also present in many samples of butter.

* See footnote, p 53.

Sodium fluoride, salicylic, and benzoic acids are also sometimes used as preservatives for these substances.

Boric acid and borax should be tested for, in the aqueous liquid which separates from butter or margarine on warming with a little water, by the methods described under milk (p. 21).

To determine the amount of boron preservative, a weighed quantity of the material is shaken with hot water and a known portion of the aqueous extract, after being made alkaline by sodium hydroxide to prevent loss of boric acid, evaporated to dryness and ignited to remove organic matter. The boric acid is then determined in the residue as described in Vol. I., p. 41.

OTHER PRODUCTS CONSISTING OF, OR CONTAINING, EDIBLE OILS AND FATS.

Lard.

This product should consist entirely of pig fat and be free from water. It is, however, sometimes adulterated by the addition of beef fat and cotton-seed oil.

The detection of adulteration of lard is a matter of some difficulty. Thus it may be noted that the *iodine value* of lard, which may be from 45 to 65, lies between that of beef fat, which has a low value of about 35, and that of cotton-seed oil, which is high, usually between 105 and 115. Whilst, therefore, the addition of appreciable quantities of *either* beef fat or cotton-seed oil could be detected by a determination of the *iodine value* of the mixture, in the former case a low value and in the latter case a high value being obtained; if *both* substances are added no evidence of adulteration would be obtained by a determination of this constant.

The addition of cotton-seed or other vegetable oil can, however, be detected by means of the phytosterol which they contain, but, as pointed out before (p. 49), if a small quantity of paraffin wax is added at the same time, the detection of the adulteration is more difficult.

Cheese.

This product is obtained from curdled milk which has been naturally or artificially soured. It consists of a homogeneous mixture of water, protein (chiefly casein), fat, and small quantities of lactose, lactic acid, and mineral matter. It

differs, of course, from butter in having a high content of protein. The characteristic flavour of cheese is developed during the process known as ripening, and is chiefly due to small amounts of substances produced by decomposition of the proteins by bacterial action. The chief varieties derive their names from towns or districts where they were first made.

Different varieties of cheese differ considerably in composition, but on the average the various constituents are present as follows :—

Water, 30 to 40 per cent.; fat, 25 to 35 per cent.; casein, 25 to 30 per cent.; ash obtained on ignition, 3 to 5 per cent.

Cheese made from skimmed milk contains practically no fat, and may contain as much as 70 per cent. of water. For further information as to the composition of cheese and cream cheese, see "The Analyst," 1924, **49**, 264-270.

Olive Oil.

This oil is used largely as a salad oil and for packing sardines, etc. It is often adulterated by the addition of cheaper oils such as cottonseed, arachis, or petroleum oils. Substitution of the last-named for olive oil might be objected to as it has no food value, petroleum oils passing through the body unchanged.

Cottonseed oil added to olive oil can be detected by the test described below.

The addition of petroleum oil has been dealt with under determination of saponification number (p. 37).

Arachis (pea-nut) oil is detected by the separation of arachidic acid, $C_{19}H_{39}COOH$, from the products of hydrolysis of the oil. This involves a large number of operations, and for further details, see " Fatty Foods," by Bolton and Revis.

Cottonseed Oil.

This oil, which has a characteristic taste and smell, is used largely as a salad oil, in the manufacture of cheap confectionery, and for frying fish. It is also used in the manufacture of margarine and as an adulterant for lard.

To test for the presence of cottonseed oil in an oil or fat, place about 2 c.c. of the substance in a test tube with an equal volume of amyl alcohol and the same volume of a solution of

sulphur in carbon bisulphide (containing 1 gm. of sulphur in 100 c.c. of carbon bisulphide). Put a plug of cotton-wool in the mouth of the test tube and heat the tube in a boiling saturated solution of common salt for about fifteen minutes in the fume-cupboard. In the presence of cottonseed oil a deep orange to red colour is produced. Cottonseed oil which has been heated very strongly or hydrogenated does not give this coloration.

In connection with this test for the presence of cottonseed oil in lard, it should be noted that the fat of pigs fed on cottonseed meal gives a similar faint coloration.

For *saponification* and *iodine values* of cottonseed and olive oils, see pages 35 and 44

Chapter III.

CARBOHYDRATE FOODS.

Although a large number of substances are classified chemically as carbohydrates, only relatively few of these are met with in the ordinary course of food analysis, and of these the most important are the following :—

Monosaccharides, *Hexoses* ($C_6H_{12}O_6$).

Dextrose or *d*-glucose * (Grape Sugar)—

$CH_2(OH)$
|
$CH(OH)$
|
$CH(OH)$
|
$CH(OH)$
|
$CH(OH)$
|
$C = O$
|
H

Laevulose or Fructose (Fruit Sugar)—

$CH_2(OH)$.
|
$CH(OH)$
|
$CH(OH)$
|
$CH(OH)$
|
$C = O$
|
$CH_2(OH)$.

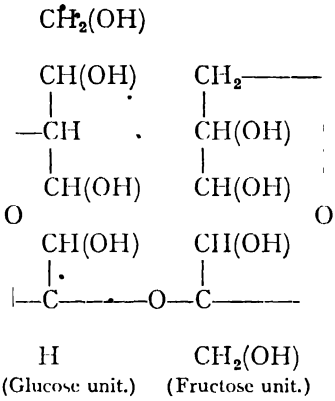
Galactose, $CH_2OH . [CH(OH)]_4 . CHO$, contains the same groupings as dextrose, but is a stereo-isomeride of this sugar, and is not identical with it.

Disaccharides, ($C_{12}H_{22}O_{11}$).

These sugars on hydrolysis give two molecules of monosaccharide $C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$.

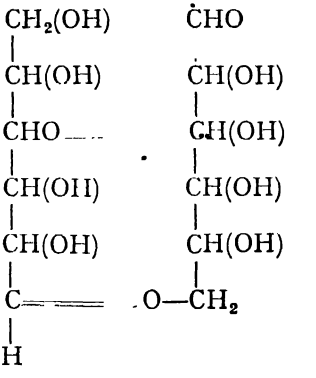
* It is advisable in speaking of this sugar to use the term "dextrose," since "commercial glucose," which is a mixture of dextrose with other substances (see p. 108), is sometimes referred to simply as glucose, and so in using this latter term confusion may arise as to whether the pure sugar or the commercial preparation is in question.

Sucrose or Cane Sugar—



(Glucose unit.) (Fructose unit.)

Lactose or Milk Sugar—



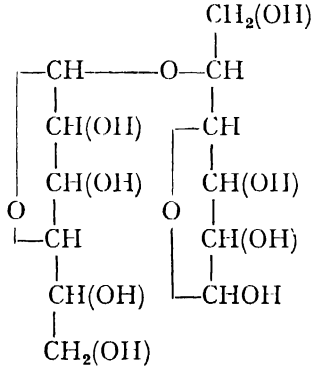
Sucrose yields on hydrolysis one molecule of dextrose and one molecule of lævulose.

Lactose yields on hydrolysis one molecule of dextrose and one molecule of galactose

Maltose or Malt-sugar, which may be represented by a formula similar to that of lactose, yields on hydrolysis two molecules of dextrose.

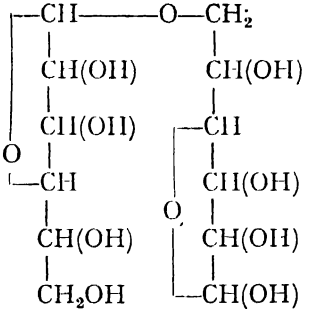
It will be seen that in the above formula for lactose (and maltose) an aldehydic group, —CHO, is present, but recent investigations indicate the formulæ which should be assigned to lactose and maltose are those given below :—

Lactose



(Galactose residue.) (Glucose residue.)

Maltose



(1st Glucose residue.) (2nd Glucose residue.)

In these formulæ the presence of a free carbonyl group is not indicated. Nevertheless, in their reactions with Fehling's solution and phenylhydrazine these sugars behave as if such a group were present.

POLYSACCHARIDES, $(C_6H_{10}O_5)_n$.

Dextrin yields on hydrolysis: dextrose.
Starch ,, ,, dextrin, maltose, dextrose.
Cellulose ,, ,, chiefly dextrose.
(See Vol. I., pp. 108, 109.)

QUALITATIVE REACTIONS OF THE MONO- AND DISACCHARIDES (SUGARS).

The substances ordinarily described as *sugars* are either mono- or disaccharides, and the reactions of the carbohydrates belonging to these two classes may be conveniently dealt with together. The reactions of the polysaccharides will be considered later (p. 98).

Reduction of Cupric Salts in Alkaline Solution or of Fehling's Solution.

Sugars containing a free carbonyl group, i.e. the monosaccharides, and also lactose and maltose, all have the power of reducing cupric salts in alkaline solution.

Sucrose which does not contain a free carbonyl group has no action on cupric salts in alkaline solution, but on boiling with dilute acid, i.e. on hydrolysis or "inversion" (see p. 71) it is converted into dextrose and lævulose, and if the solution *is then made alkaline* reduction of the copper solution is readily effected.

Dissolve a small quantity of dextrose in water, add a few drops of copper sulphate solution and then caustic soda until a clear blue solution is obtained. It should be noted that, in the presence of the sugar, cupric hydrate is not precipitated on the addition of caustic soda to the solution containing copper sulphate, but a deep blue solution is obtained. On boiling, a reddish-brown precipitate of cuprous oxide is formed. The test may also be made by using equal parts of Fehling's solution I. and II. (see p. 73). Repeat the ex-

periment with lævulose, lactose, maltose, and sucrose respectively, and observe that reduction takes place in all cases except the last.

To another portion of the sucrose solution add a few drops of hydrochloric acid and boil for about five minutes. Make the solution alkaline with sodium carbonate, then add copper sulphate and caustic soda, or Fehling's solution, as before, and boil. Cuprous oxide is precipitated.

Reduction of Cupric Acetate by Dextrose and Laevulose. (Barfoed's Test.)

Although both the monosaccharides and the disaccharides maltose and lactose will reduce cupric salts in alkaline solution, cupric acetate solution is reduced only by the monosaccharides, and this may be used as a method of distinguishing between the two classes of carbohydrates.

The reagent is prepared by dissolving 13·3 gms. of neutral, crystallised cupric acetate in 1 per cent. acetic acid solution, and diluting to 200 c.c. with the same solvent. A portion of the reagent, when heated on a water bath, should show no reduction.

In carrying out the test, 5 c.c. of the reagent are added to 5 c.c. of the sugar solution, and the mixture heated in a boiling water bath for three minutes.

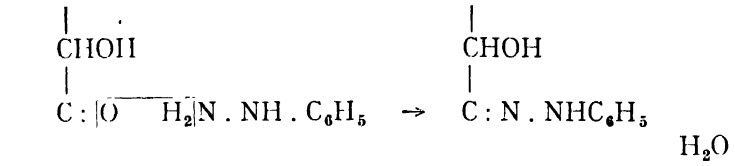
In the presence of dextrose or lævulose cuprous oxide will be precipitated.

If the heating is continued for more than three minutes, any disaccharide present may be hydrolysed.

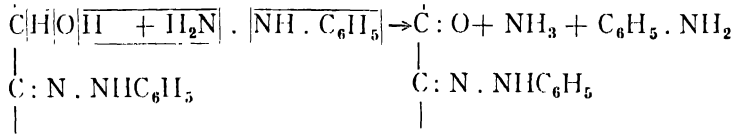
Formation of Osazones.

Sugars which are capable of reducing cupric salts in alkaline solution also react with phenylhydrazine with the formation of osazones. These osazones crystallise in characteristic forms, and may be readily identified with the aid of the microscope.

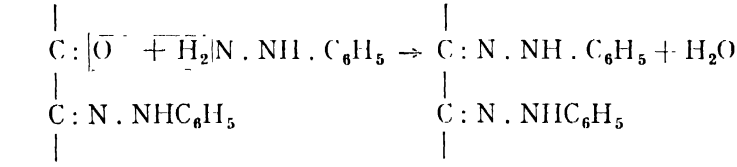
On treating a carbohydrate containing the grouping —CH(OH)—CO— with phenylhydrazine, a hydrazone is first formed by the interaction of the phenylhydrazine with the carbonyl group, water being eliminated.



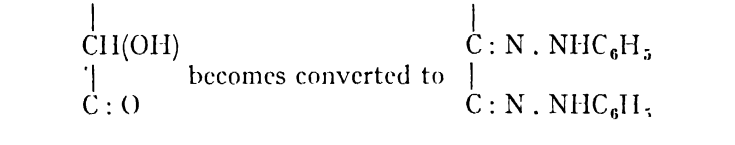
A second molecule of phenylhydrazine then reacts with the grouping — CHOH, which by the elimination of two atoms of hydrogen becomes converted into a carbonyl group, with the production, at the same time, of aniline and ammonia.



This carbonyl group reacts with a third molecule of phenylhydrazine, as in the first stage of the reaction.



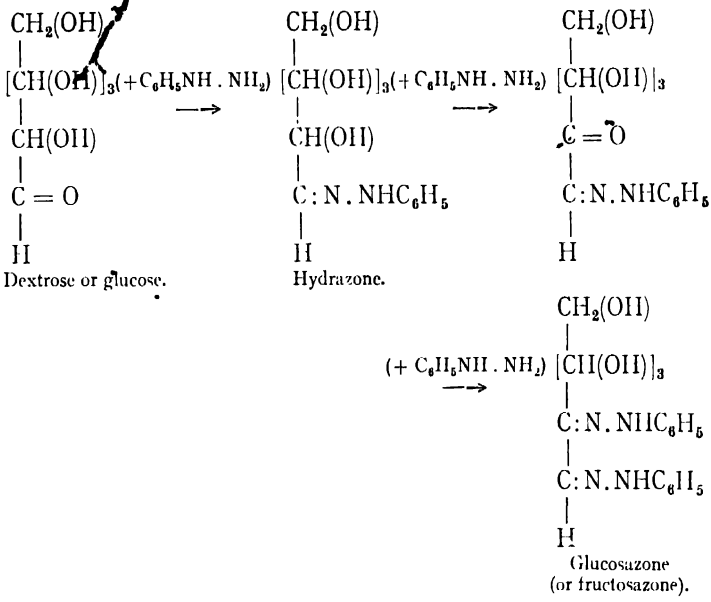
so that finally the grouping—



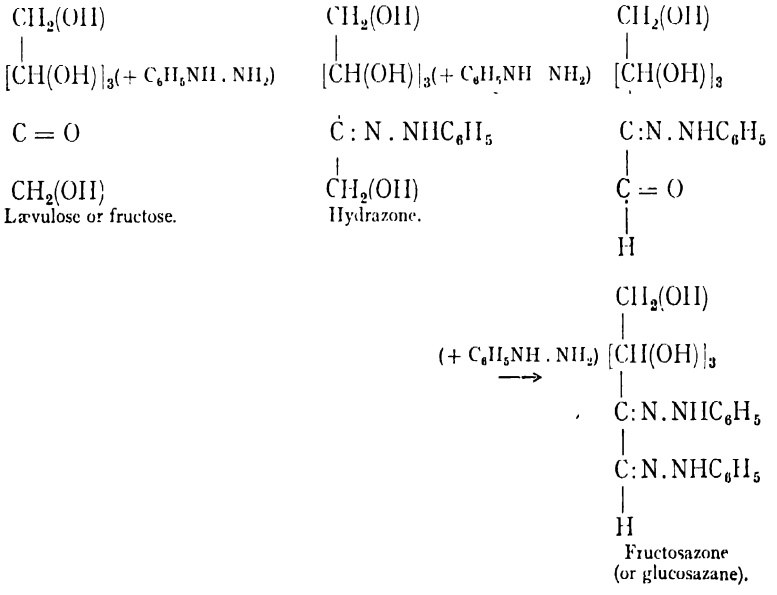
and this is the group characteristic of the osazones.

Glucosazone and Fructosazone.—The osazones obtained from dextrose and lævulose are identical. This indicates that in each case it is a (—CHOH—) group adjacent to the carbonyl group which becomes oxidised during the second stage of the reaction. The formation of the same osazone from both dextrose and lævulose may be shown in the following manner :—

Glucose—



Fructose.—



To Prepare an Osazone.

Mix a small quantity of phenylhydrazine hydrochloride (about 0·2 gm.) with twice its weight of sodium acetate, and dissolve in 10 c.c. of water, filter if not quite clear, and add a few drops of acetic acid to the filtrate. The sodium hydroxide formed by the hydrolysis of the sodium acetate will liberate the base, phenylhydrazine, from its hydrochloride.

To this solution add about 5 c.c. of the sugar solution under examination (concentration preferably about 2 per cent.), and heat in a test tube in a boiling water bath from half an hour to an hour.

The osazone separates out as a yellow precipitate which is more or less crystalline in character.

Glucosazone separates from the hot solution, but the osazones of the disaccharides maltose and lactose separate only on cooling, and it is possible in the case of mixtures containing both a mono- and a disaccharide to separate the osazones almost completely by fractional crystallisation. The osazone which separates from the hot solution is filtered rapidly through a small filter paper and washed with hot water. On cooling, the osazone of the disaccharide separates out from the filtrate.

Prepare specimens of glucosazone, lactosazone, and maltosazone by the above method, and in each case examine the precipitate under the microscope. If the precipitate does not show any crystalline structure, i.e. is amorphous, it may be recrystallised from alcohol and water. Dissolve the precipitate in a small quantity of warm alcohol, add an equal volume of water, and boil to drive off the alcohol. On cooling, the osazone will separate out in a crystalline form. The difference in crystalline structure of the three osazones is then readily apparent.

Glucosazone forms fine needle-shaped crystals which separate out in clusters resembling wheat sheaves (see Plate I. *A*).

Lactosazone separates as small nodular particles, which when examined under the microscope are seen to be composed of fine needle-shaped crystals, and the groupings resemble burrs in appearance (see Plate I. *B*).

Lactosazone can also be prepared from milk after the removal of the proteins and fat (see p. 15).

Maltosazone forms broad needle-shaped or spatula-like crystals which are usually grouped together in the form of rosettes (see Plate I. *C*).

PLATE I

MICROPHOTOGRAPHS OF OSAZONES

(Magnification—approximately 240)

A. Glucosazone

B. Lactosazone

C. Maltosazone

[*To face p.* 70

Melting-points of Osazones.—The osazones are sometimes distinguished from one another by means of their melting-points. For this purpose, however, they require to be carefully purified, as the differences in melting-point are not very great :—

Glucosazone	M.P. = 204-205° C.
Lactosazone	,, = 200° C.
Maltosazone	,, = 206° C.

Optical Activity.

This subject is dealt with more fully under the "Quantitative Examination of the Sugars" (see p. 76), the apparatus used and the methods employed being there described, but the following observations will serve to indicate that these optical properties may be utilised as a means of differentiating the principal sugars, apart from any determination of the amount present. All the naturally occurring sugars are optically active, i.e. possess the power of rotating the plane of polarised light. Some of the sugars are dextro-rotatory, or have a positive rotation, and some of them are lævo-rotatory or negative, and this in some instances forms a convenient method of distinguishing them. Thus dextrose and lævulose, which both reduce cupric salts in alkaline solution, and which form the same osazone, can readily be distinguished by their behaviour towards polarised light, dextrose being dextro-rotatory and lævulose lævo-rotatory, hence the names dextrose and lævulose.

Observations of the rotation of the plane of polarised light produced by a sugar before and after hydrolysis are also often of value in indicating the nature of the sugar present.

Sucrose, which is dextro-rotatory, yields on hydrolysis one molecule of dextrose and one molecule of lævulose from each molecule of sucrose. The lævo-rotation by the lævulose is distinctly greater than the dextro-rotation of the dextrose (see p. 84), hence the effect of the hydrolysis is to change or *invert* the rotation from positive to negative, and it is for this reason that the term *inversion* is applied to the hydrolysis of sucrose, and that the equimolecular mixture of dextrose and lævulose thus produced is described as *invert sugar.*

Both maltose and the dextrose formed from it by hydrolysis

are dextro-rotatory, but the rotation produced by the maltose is greater than that produced by the dextrose, and the effect of hydrolysis is to decrease the angle through which the plane of polarised light is rotated (see p. 84). The rotation, however, remains positive throughout.

In the case of lactose, on the other hand, there is a slight increase in the amount of rotation produced on hydrolysis, since the equimolecular mixture of dextrose and galactose, produced by its hydrolysis, rotates the plane of polarised light through a slightly greater angle than the lactose (see p. 84). In this case, also, the sign of the rotation is unaltered by hydrolysis.

QUALITATIVE EXAMINATION OF THE SUGARS.

The mono- and disaccharides when obtained singly and in a pure condition can readily be identified by means of the reactions described above, but before applying these methods to the examination of food products it is necessary to separate the sugars from the other constituents in a comparatively pure condition, otherwise erroneous results may be obtained. For example, the reduction of alkaline copper sulphate solution is not peculiar only to the sugars. Also in cases where several sugars may possibly be present it is difficult to obtain exact information as to the nature of these sugars by means of qualitative reactions alone, and more complete information on this point is best furnished by a careful interpretation of the results of a quantitative analysis.

On this account the methods of examining typical sugar products will be dealt with later, after consideration of the quantitive methods used for the determination of the sugars.

QUANTITATIVE ANALYSIS OF THE SUGARS.

The principal quantitative methods used for the examination of the sugars are based on their power of reducing Fehling's solution and on their optical activity.

In dealing with the individual sugars these methods may be regarded as alternative, but in the case of mixtures containing two or more sugars it is usually necessary to combine the two methods in order to obtain the proportions of the different sugars present.

Quantitative Determination of the Sugars by Fehling's Solution (Volumetric method).*

Preparation of the Solution.—Fehling's solution is prepared in two parts :—

Solution I. contains 69·278 gms. of pure crystallised copper sulphate, $CuSO_4 . 5H_2O$, dissolved in water and diluted to 1 litre.

Solution II. contains 346 gms. of Rochelle salt (sodium potassium tartrate,

$$KOOC . CH(OH) . CH(OH) . COONa . 4H_2O),$$

and 142 gms. of sodium hydroxide dissolved in 1 litre of water.

On mixing the two solutions a clear deep blue solution is obtained. The presence of the Rochelle salt prevents the precipitation of the cupric hydrate by the caustic soda. The solution should remain clear even after boiling for some minutes.

In using the reagent, 5 c.c. of solution I. are mixed with 5 c.c. of solution II.† and the solution diluted with 30 c.c. of water.

The mixture is heated to boiling-point in a porcelain dish or flask, and the sugar solution run in from a burette until the copper salt is completely reduced to cuprous oxide.

The weight of any sugar which is required to bring about this reduction is usually termed its *copper-reducing power.* The copper-reducing powers of the more important sugars are given below :-

Copper-Reducing Powers.

10 c.c. of Fehling's solution (5 c.c. of No. I. and 5 c.c. of No. II.) are reduced by :—

Dextrose Lævulose Invert sugar	. .	= 0·05 gm.
(Sucrose	. .	= 0·0475 ,,)
Maltose	. .	= 0·0807 ,,
Lactose (anhydrous) ‡	.	= 0·0642 ,,
Starch (after hydrolysis)		= 0·045 ,,

* For details of gravimetric processes for the determination of sugars by means of Fehling's solution see "Food Inspection and Analysis," by Leach.

† In quantitative work it is, of course, necessary to measure out these quantities with pipettes, using separate pipettes for the two solutions.

‡ See also p. 16.

As already stated, sucrose does not reduce Fehling's solution until converted into invert sugar (see p. 66). The quantity of invert sugar required to reduce 10 c.c. of Fehling's solution is 0·05 gm., and this amount of invert sugar is produced by the hydrolysis of 0·0475 gm. of sucrose: This may be shown by consideration of the equation—

$$\underset{342}{C_{12}H_{22}O_{11}} + \underset{18}{H_2O} \rightarrow 2\underset{360}{C_6H_{12}O_6}.$$

Thus 360 gms. of invert sugar are obtained from 342 gms. of sucrose, or 0·05 gm. of invert sugar is obtained from—.

$$\frac{342}{360} \times \cdot 05 = 0{\cdot}0475 \text{ gms. sucrose}$$

The Standardisation of Fehling's Solution.

For accurate work it is advisable to standardise the Fehling's solution before use. This may be done by means of a solution of pure dextrose of known concentration.

Weigh out accurately 0·5 gm. of pure dextrose, dissolve in distilled water, and make up the volume to 100 c.c. Fill a burette with some of this solution and pipette out into a conical flask or porcelain basin 5 c.c. of Fehling's solution I. and 5 c.c. of Fehling's solution II., dilute with 30 c.c. of water, and boil. Proceed as described in the determination of lactose (see p. 15), using potassium ferrocyanide as an outside indicator.

Method of Calculation.—Theoretically 10 c.c. of the dextrose solution (= 0·05 gm.) should be required for the reduction of the Fehling's solution. If this result is not obtained on titration, the exact amount of dextrose required for the reduction of the Fehling's solution should be calculated and recorded for future use, or a factor for the solution may be employed.

Example.—If the mean of three titrations shows that 9·6 c.c. of the dextrose solution (0·5 gm. of dextrose in 100 c.c.) are required to reduce 10 c.c. of Fehling's solution, then since

1 c.c. of dextrose solution contains 0·005 gm. dextrose,
9·6 c.c. ,, ,, ,, 0·005 × 9·6 gm. dextrose
= 0·048 ,, ,,

Hence in this case 0·048 gm. of dextrose is required to reduce

10 c.c. of the Fehling's solution, or 0·05 gm. of dextrose would reduce—

$$\frac{10 \times 0{\cdot}05}{0{\cdot}048} = 10{\cdot}4 \text{ c.c. of the given Fehling's solution.}$$

So that if from the volume of a sugar solution, found by titration to reduce 10 c.c. of the given Fehling's solution, the volume which would be required to reduce 10·4 c.c. of the Fehling's solution is calculated, this gives the volume of sugar solution which contains 0·05 gm. of dextrose or its equivalent.

In the case of the Fehling's solution under consideration, when used for determination of sugars, the volume of the sugar solution used $\times \frac{10{\cdot}4}{10}$ (i.e. 1·04) would give the required volume of the solution which contained 0·05 gm. of dextrose (or its equivalent).

Determination of Sucrose by Fehling's Solution.

For practice in carrying out inversion and dealing with invert sugar solutions, a determination of the amount of sucrose present in a sample of ordinary cane sugar (lump or white granulated sugar may be used) should be made with Fehling's solution, and the result afterwards checked by making a polarimetric determination on the same sample (see p. 85).

Prepare a 1 per cent. solution of the sugar by diluting 10 c.c. of the 10 per cent. solution prepared for the polarimetric determination (see p. 92) to 100 c.c. with water.

Measure out 50 c.c. (= 0·5 gm. sugar) of this solution into a clean flask, add 5 c.c. of concentrated hydrochloric acid, and heat on a boiling water bath for ten minutes, to convert the sucrose into invert sugar. Cool and add solid sodium carbonate to the solution until it is distinctly alkaline. Transfer the solution to a graduated flask and make up the volume to 100 c.c.

100 c.c. of this solution now contain invert sugar equivalent in amount to the sucrose originally present in 0·5 gm. of the sugar.

Fill a burette with this sugar solution and determine the volume of the solution required to reduce 10 c.c. of Fehling's solution (see p. 74).

This gives the volume of the solution which is equivalent to 0·0475 gm. sucrose (see p. 73), and from this the amount

of sucrose present in 100 c.c. of the solution can be calculated, i.e. the amount of sucrose present in 0·5 gm. of the sugar taken; and hence the percentage of sucrose in the sample obtained.

Example.—50 c.c. of a 1 per cent. solution of cane sugar were inverted, the solution made alkaline and diluted to 100 c.c.

9·8 c.c. of this diluted solution were required to reduce 10 c.c. of Fehling's solution.

Since the copper-reducing power of sucrose = 0·0475 gm. (i.e. 10 c.c. of Fehling's solution are reduced by 0·0475 gm. sucrose when converted into invert sugar), it follows that—
9·8 c.c. of diluted sugar solution (0·5 gm. in 100 c.c.) are equivalent to 0·0475 gm. sucrose,
or 100 c.c. of diluted sugar solution are equivalent to

$$\frac{0{\cdot}0475}{9{\cdot}8} \times 100 \text{ gms. sucrose.}$$

Thus 0·5 gm. of sugar contains $\frac{0{\cdot}0475}{9{\cdot}8} \times 100$,, ,,

or 100 ,, ,, ,, $= \frac{0{\cdot}0475}{9{\cdot}8} \times \frac{100 \times 100}{0{\cdot}5}$,,

$= 96{\cdot}9$ gms. of sucrose.

The sugar contains 96·9 per cent. of sucrose.

Determination of Lactose and Maltose.

The determination of lactose in milk has already been dealt with (see p. 15). The quantity of maltose present in a solution of this sugar can also readily be obtained by the methods just described on the basis that 0·0807 gm. of maltose is required for the reduction of 10 cc.. of Fehling's solution.

Polarimetry of the Sugars.

The Polarisation of Light.

Before dealing with the subject of polarimetry and polarimetric examination of the sugars, it is necessary to discuss briefly the properties of polarised light and the methods usually employed for the production and examination of rays of polarised light.

Light is produced by vibrations in the ether, and in the case of ordinary light these vibrations are executed in all

directions in a plane at right angles to the direction of propagation of the light. This point will perhaps be more clearly understood by taking a mechanical illustration.

Ordinary light may be represented by a wheel travelling *in the direction of* its axle. In this case the vibrations composing the light will be executed along any or all of the spokes, i.e. along any line drawn through the centre of the wheel to its circumference, since all these lines are in a plane at right angles to the axle of the wheel, and hence to the direction of propagation.

When a ray of light strikes a piece of glass or other transparent medium it is deflected from its original direction according to the laws of refraction, but the vibrations are still executed in all directions in a plane at right angles to the direction in which the light is then travelling. When, on the other hand, a ray of light strikes certain crystalline substances, e.g. tourmaline or Iceland spar, it is split up into two distinct rays or undergoes *double refraction*.

One of these rays obeys the ordinary laws of refraction, and is known as the *ordinary ray*: the other, which does not obey these laws is known as the *extraordinary ray*. Further, it may be shown that although the light is apparently unaltered in character, the vibrations which constitute these rays are not executed in *all* directions in a plane at right angles to the direction of propagation, but only in *one particular direction* in this plane. The light is therefore said to be *plane polarised*.

Or, referring again to the mechanical illustration of a wheel and axle, in the case of polarised light the vibrations will be executed along one of the spokes of the wheel (and the spoke opposite to it) only, i.e. along one particular diameter of the wheel.

As the wheel moves along in the direction of its axle this diameter will trace out a plane, and this plane corresponds to the *plane of polarisation*.

The effects of polarisation may also be roughly illustrated by a model.

If a string be stretched between two points A and D, as in Fig. 9, and then caused to vibrate, the vibrations will be free to occur from side to side, upwards and downwards, or in any intermediate position, i.e. in any direction in a plane at right angles to the length of the string. If, however, a disc B with a vertical slit is placed on the string, the vibrations

will be executed upwards and downwards only, i.e. in direction parallel to that of the slit, since any motion from side to side will be stopped by the edges of the slit (see Fig. 9).

The model is in one respect imperfect, since the vibrations

FIG. 9.

are polarised before reaching the slit instead of being polarised only after passing through the slit. For the purpose of illustration, however, it may be assumed that until the string reaches the slit it is free to vibrate in all directions perpendicular to its length.

Method of Producing Polarised Light.

The two rays produced by double refraction, although both polarised, are not polarised in the same plane, but in two planes at right angles to each other, i.e. the vibrations which constitute these rays are executed in two fixed planes at right angles to each other.

In order to produce light which is polarised in one plane only, a device known as a *Nicol prism* is used.

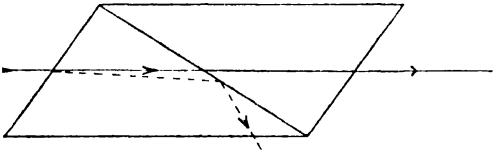


FIG. 10.—Nicol Prism.

A Nicol prism consists of a rombohedron of Iceland spar divided into two by a section through its obtuse angles. The cut surfaces are polished and cemented together again in their original position by a layer of Canada balsam, the surface covered by the Canada balsam being spoken of as the *principal section* of the prism (see diagram, Fig. 10). Light falling on one face of the prism is split up into an ordinary and extraordinary ray, but on reaching the layer of Canada balsam the

ordinary ray undergoes total reflection, as indicated by the dotted line. The extraordinary ray passes on and emerges at the opposite face of the prism. There is nothing to render the peculiar condition of the ray emerging from a Nicol prism visible to the naked eye, but if the ray is viewed through a second Nicol, called the *analyser*, it is possible to detect the fact that the light is polarised.

The part played by the analyser may be illustrated by again referring to the model described above (Fig. 9).

If the string is made to vibrate so that the vibrations travel from left to right, then, as already stated, the string is theoretically free to vibrate in all directions at right angles to its length as far as the slit *B*. After passing this slit, however, the vibrations are executed in a vertical direction only.

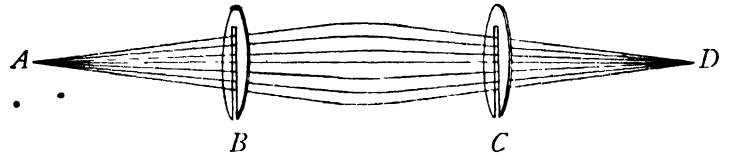


FIG. 11A.

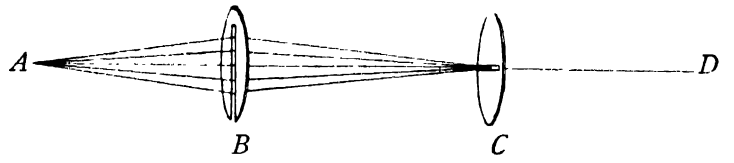


FIG. 11B.

If a second vertical slit be placed to the right of the first the vibrations will be free to pass through this slit also (Fig. 11A); but if, as in Fig. 11B, the second slit be placed in a horizontal position, the vibrations will be stopped on reaching the second slit, and the string between this slit and *D* will be at rest.

A may be taken to represent a source of light, and the vibrations of the string which represent the vibrations which constitute this light are polarised by a Nicol prism represented by *B*. Hence they are then executed along one direction only, and if the second prism or analyser represented by *C* is placed with its principal section parallel to that of the first, the vibrations will pass through the second Nicol, and light will reach the eye of an observer placed at *D*.

If, on the other hand, the second Nicol is turned with its principal section at right angles to that of the first, the vibrations produced by the first Nicol will be extinguished by the second Nicol, and no light will reach the eye. In this position the Nicols are said to be *crossed*. In intermediate positions the light will be partially transmitted through the analyser.

It must be clearly understood that the Nicol prism contains no actual slits, but its effect on the vibrations which constitute light may be compared to the action which slits in a diaphragm would have on vibrating particles of more material size.

Optical Activity.

When a ray of polarised light passes through a layer of certain organic substances either in the liquid form or in solution, it is found that the vibrations which constitute the emerging ray are no longer executed in the same plane as those of the incident ray, but in a plane inclined at some angle to it.

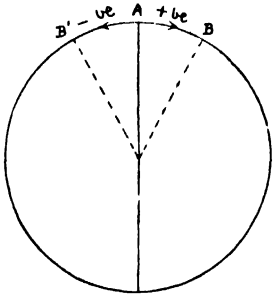


FIG. 12.

Thus, if a layer of such a substance be inserted between two Nicols placed with their principal sections parallel to one another, it will be found that light is only partially transmitted through the analyser, and in order to obtain the position of maximum transmission of light the analyser must be rotated either towards the right or towards the left. On the other hand, if the Nicols were originally crossed, it will be found that when a layer of such a substance is inserted between them some light is transmitted through the analyser, and in order to obtain again complete extinction of the light, the analyser must be rotated through an angle either towards the right or towards the left. Substances which have this power of *rotating the plane of polarisation of light* are said to be *optically active*. Those which show the phenomenon in solution usually contain one or more asymmetric carbon atoms, i.e. atoms to which four different atoms or groups are attached (see p. 64). Certain solids, however, such as quartz, SiO_2, are also optically active.

Optically active substances may be classified according to the direction in which the plane of polarisation is rotated; thus, if when looking along the direction in which the light is transmitted, and *towards* the source of the light, the plane of polarisation appears to be rotated in the direction in which the hands of a clock revolve, the substance is said to be *dextro-rotatory*, or to have a *positive* rotation; whereas when the plane of polarisation appears to be rotated in a contra-clockwise direction the substance is said to be *lævo-rotatory*, or to have a *negative rotation.*

Thus, in Fig. 12, if a ray of light is emerging from the paper in a direction perpendicular to the plane of the paper the rotation will be positive when it is in the direction AB, and negative when it is in the direction AB'.

The Polarimeter.

In order to measure the angle, through which the plane of polarisation has been rotated by an optically active substance, an apparatus called a polarimeter is used.

The polarimeter in its simplest form consists of two Nicol prisms; one of these is fixed and is used to produce polarised light; it is therefore called the *polariser.* The second acts as analyser, and is so arranged that it can be rotated through any desired angle either towards the right or towards the left.

If a layer of an optically active substance is introduced between the two Nicols when they are crossed, the angle through which the analyser has to be rotated, in order again to obtain complete extinction of the light, will give a measure of the angle through which the plane of polarisation has been rotated.

For accurate measurements, however, such an arrangement is not sufficiently sensitive, since it will be found that when the analyser is adjusted to the position of complete extinction of the light, it can be rotated through a small angle in either direction without the passage of light being perceptible. Polarimeters are, therefore, usually fitted with some device for increasing the sensitiveness of the apparatus.

Laurent's Half-shadow Polarimeter.—In the Laurent half-shadow polarimeter, which is one of the most frequently used types, and which is in use in this laboratory, a circular plate, one-half of which is composed of quartz and the other of

ordinary glass, is placed between the polariser and the tube containing the optically active substance.

The effect of the quartz plate is to rotate the plane of polarisation of the light which falls upon it through an angle. So that when the field is examined with the analyser turned parallel to the polariser, that half of the field which is covered by the glass will be seen at its position of maximum illumination, whilst only a portion of the light transmitted through the quartz plate will enter the analyser and the two halves of the field are therefore unequally illuminated. If the analyser is turned until the quartz plate is seen at its position of maximum illumination the field will again be unequally illuminated, since in this case only a portion of the light transmitted through the glass plate will enter the analyser. Between these two extreme positions there is one position of the analyser in which the two halves of the field are equally illuminated; namely, when the analyser is turned so that it will transmit completely vibrations executed in a plane, which is equally inclined to the planes of polarisation of the light transmitted through the two halves of the plate. This is *the zero position of the instrument.*

If the analyser is adjusted to the zero position, and a layer of an optically active substance then inserted between the polariser and analyser, the two halves of the field will no longer be uniformly illuminated. In order again to obtain the position of uniform illumination the analyser will have to be rotated through an angle either to the right or to the left; this angle will be equal in magnitude to the angle through which the plane of polarisation has been rotated by the optically active substance.

For further details of the theory of polarised light, reference may be made to standard text-books on Physics.

Specific Rotatary Power.

The magnitude of the angle, through which the plane of polarisation of light is rotated by a solution of an optically active substance, depends not only on the quantity of optically active substance present, i.e. on the concentration of the solution, but also on the depth of liquid through which the light travels. Thus the rotation produced by a solution of an optically active substance can be doubled, without altering the concentration of the solution, by allowing the light to traverse a layer of the solution twice the depth of

the original layer. The specific rotatory power of a substance is therefore defined as *the rotation, measured in angular degrees of the plane of polarised light produced by a solution of the optically active substance having a concentration of* 1 *gm. of the substance in* 1 *c.c. of water (or other solvent) and measured in a* 1*-decimetre tube.*

In order to obtain the specific rotatory power of a substance it is only necessary to observe the rotation produced by a solution of known concentration when measured in a tube of known length. Since if a = rotation observed by a solution of the substance containing w gms. per c.c. measured in a tube l decimetres long, then the specific rotatory power (S) may be calculated in the following manner :—

w gms. of substance in 1 c.c. give a rotation a in a tube of l dm.

1 " " " " " $= \frac{a}{w}$ " "

In a tube l dm. long 1 gm. in 1 c.c. gives a rotation $= \frac{a}{w}$

" " 1 " " " " " " $= \frac{a}{w \times l}$

Or $$S = \frac{a}{w \times l}$$

Conversely, if S is known the value of w may be calculated from the above expression, which gives—

$$w = \frac{a}{S \times l}$$

The specific rotatory power of a substance depends on the nature of the light used, and for the type of polarimeter here described monochromatic light must be used. The symbol $[\alpha]_D$ is usually employed to indicate the specific rotatory power of a substance when observed with the light from a sodium flame. The D indicates the D line of the yellow portion of the spectrum.

In many cases it is more convenient to express the concentration of the solution in grams per 100 c.c. instead of in grams per c.c. ; so that if c represents the number of grams of the substance present in 100 c.c. of the solution, $\frac{c}{100}$ may be

substituted for w in the above expression, which then becomes

$$[\alpha]_D = \frac{a}{l \times \frac{c}{100}} = \frac{100a}{l \times c}.$$

This gives the expression in the form in which it is most frequently employed in making polarimetric determinations.

It is found that the specific rotatory power of a sugar solution varies slightly with the degree of concentration of the solution, i.e. the specific rotatory power calculated from observations made with dilute solutions (e.g. 2 to 3 per cent.) does not in all cases agree exactly with that calculated from observations made with more concentrated solutions (e.g. 20 to 30 per cent.). It is therefore advisable to work always with solutions of approximately the same concentration, and a 10 per cent. solution is most generally employed.

In some cases also, notably lævulose, the specific rotatory power varies with the temperature. The temperature at which the rotation is measured may be indicated by using the symbol $[\alpha]^t_D$, where t is temperature in degrees centigrade.

For the purposes of reference the specific rotatory powers of the principal sugars are given below :—

The Specific Rotatory Powers of the Sugars.

(*Measured on a* 10 *per cent. solution.*)

Sucrose	$[\alpha]^{15°}_D = +\ 66{\cdot}5°$
Invert sugar	$= -\ 22{\cdot}7°$ (see p. 85).
Maltose	$= +\ 138{\cdot}5°$
Lactose	$= +\ 52{\cdot}5°$
Dextrose	$= +\ 52{\cdot}5°$
Lævulose	$= -\ 98{\cdot}0°$ (see p. 95).
Dextrin	$= +\ 200°$

Dextrin is not a sugar, but frequently occurs in sugar products, and its specific rotatory power is given here for the sake of completeness.

It should be noted that there is considerable discrepancy in the values given for the specific rotatory power of lævulose by different observers. Solutions of this sugar exhibit the phenomenon of muta-rotation (see p. 96), and the specific

rotatory power is very considerably influenced by changes in temperature and concentration.

For the purpose of the determinations here described, the value $-98{\cdot}0°$ at 15° C. given above may be used, and a correction made (see p. 95) for observations made at other temperatures.

This variation with the temperature will also affect the specific rotatory power of invert sugar, since the specific rotatory power of this sugar may be taken as the algebraic mean of the specific rotatory powers of dextrose and lævulose,

i.e. $\frac{-98 + 52{\cdot}5°}{2} = -22{\cdot}7°$.

Polarimetric Determination of Cane Sugar.

The above considerations show that the quantity of any *one* sugar present in a solution may be readily determined from observations of the amount of rotation of the plane of polarised light, produced by a measured length of the solution, assuming that the specific rotatory power of the sugar is known.

The apparatus used for this purpose is the Laurent half-shadow polarimeter, the principle of which has already been described (see p. 81).

The instrument should preferably be used in a dark room, but if this is not available an opaque focussing cloth should be used when observing the illumination of the field.

A diagram of this instrument and a plan of its cross-section are shown in Figs. 13 and 14. The position of the analyser and polariser are indicated by the letters *F* and *B* respectively. These are separated by a trough with a hinged lid, in which is placed the tube containing the liquid under examination. The analyser and attached eye-piece are fixed to the disc *T* (Fig. 13), which may be rotated in either direction. Round the disc is engraved a circular scale, by means of which, together with the verniers, the angle through which the analyser is rotated may be measured.

A sodium flame is produced by means of a specially constructed Bunsen lamp, fitted with a spoon, and the light is directed towards the polariser.

The polarimeter tubes are cylindrical glass tubes fitted at either end with a brass screw cap (Fig. 15). By means of this cap and a rubber washer, a small glass plate which just fits the

end of the tube is held tightly in place against the end of the tube, thus rendering the tube watertight. The length of the tube is obtained by removing both the caps and measuring the length of the glass tube.

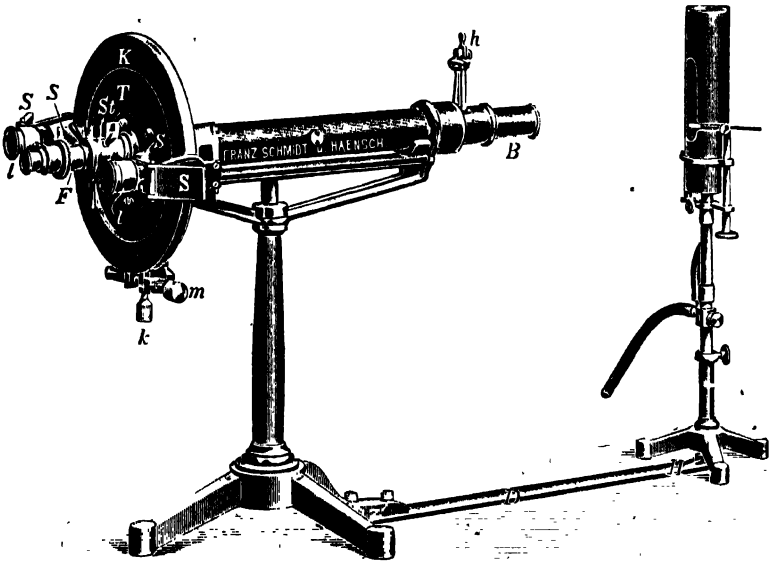


FIG. 13.—Polarimeter.

Three tubes are usually supplied with each instrument, of length 1 decimetre, 2 decimetres, and 2·2 decimetres respectively. The 2-decimetre tube is the one most usually employed, but in cases where observations have to be made with solutions which are not quite clear, or which are slightly

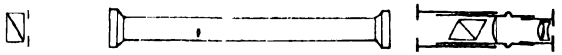


FIG. 14.—Section of Polarimeter.

coloured, better results may be obtained by using the 1-decimetre tube, since the intensity of the light transmitted through a depth of 2 decimetres may be insufficient to enable satisfactory observations to be made.

The 2·2-decimetre tube is supplied for taking observations

with solutions of invert sugar, and its use will be described later (see p. 94).

To fill a polarimeter tube proceed as follows. After having measured and cleaned the glass tube, close one end by means of the glass plate provided, place the washer on the top of the plate,* and screw on one of the brass caps.

Rinse out the tube twice with a small volume of the liquid to be examined, and then fill the tube *completely* with the solution, i.e. the meniscus of the solution should stand slightly above the edge of the tube, and any bubbles of air should be allowed to escape.

Slide the second glass plate gently over the end of the tube, so as to close the tube without introducing any air. Place the washer in position and screw on the cap.

If this operation is carefully carried out the tube should contain no air, neither should it leak; but if on tilting the tube into a horizontal position bubbles of air are observed the operation must be repeated. With tubes which have one

FIG 15. Polarimeter Tube.

end slightly enlarged, as shown in Fig. 15, it is not essential to avoid the presence of an air bubble, for when the tube is placed in a horizontal position the bubble will rise into the expanded portion of the tube and thus be out of the line of vision. Tubes having a small bulb blown in the glass into which the bubble can be driven can also be employed. If the tube shows any tendency to leak the ends may be *very slightly* greased.

Before placing the tube in the polarimeter *carefully wipe both ends* with a clean cloth to remove any moisture adhering to the outer surfaces of the glass plates. Difficulties in focusing experienced by the beginner are often due to neglect of this precaution.

The Polarimeter Scale.

Polarimeters are usually fitted with a circular scale graduated in angular degrees from 0° to 360°.

* The mistake must not be made of putting the washer between the end of the tube and the plate.

In some cases, however, instead of or in addition to this scale, the instrument is provided with a sugar scale.* This latter is a scale of 100 degrees so arranged that when a specific quantity of cane sugar is used for the determination the number of degrees indicated by the scale represents the percentage of pure sucrose without any calculation.

Instruments fitted with this form of scale only, should, strictly speaking, be described as *saccharimeters*, since they can only conveniently be employed for cane sugar solutions. For general utility, and for the purpose of acquiring some general knowledge of polarimetric methods, the angular scale is to be preferred, and is the one which will be considered here.

(For further information as to the methods of using the different forms of saccharimeters the student is referred to "Food Inspection and Analysis," by Leach, or any other standard work on food analysis.)

On the dial of the instrument in use in this laboratory there is engraved a circular scale of 360°, and each degree is divided into four sub-divisions, so that each sub-division represents $\frac{1}{4}$°. The zero is on the right hand side of the scale, and the degrees read upwards and towards the left round to 360.

By means of the handle provided for this purpose (see Fig. 13) the scale, together with the analyser and eye-piece, may be rotated in either direction, fine adjustments being made by means of the milled head-screw *m*. The scale should not be touched with the fingers, as fingering tends to obliterate the graduation marks, and makes it difficult to read the scale. In the instrument shown in Fig. 13 the scale is protected by a casing *K*, only that portion of it which is opposite the vernier being visible. The position of the scale is read by means of one or other of the two *fixed* verniers fitted on either side of the scale, and in order to ensure accuracy in taking the reading the magnifying lenses *ll* are provided to render the graduation marks more clearly visible (see Fig. 13).

The zero on the vernier is the fixed point from which all readings are taken.

Reading of the Vernier.—Readings are usually taken from the right hand side, and in order to avoid unnecessary confusion it may be assumed, unless otherwise stated, that the right hand vernier is used. The reading given by the left hand vernier will, of course, differ from that given by the

right by 180°. If the analyser is turned through 180° the readings will be interchanged, and it will be seen that for any position of the analyser there will always be two possible readings, differing from each other by 180°.

The vernier is graduated so that 25 vernier divisions correspond to 24 sub-divisions on the scale, and since each of these sub-divisions represents $\frac{1}{4}$ of a degree, the scale may be read by means of the vernier to

$\frac{1}{25}$ of $\frac{1}{4}$ of a degree $= \frac{1}{100}$ of degree, or ·01°.

Thus, supposing the zero on the vernier lies between 3° and 4° on the scale and between the first and second sub-division this indicates that the reading is greater than 3·25° and less than 3·5°. To obtain the reading more exactly, look along the vernier and see which graduation of the vernier coincides exactly with one of the scale divisions. Supposing that this is the graduation marked 12 on the vernier; this indicates that $\frac{12}{100}$, or 0·12°, must be added to the *lower* limit of the observed scale reading, which gives 3·25 + ·12 = 3·37° as the correct reading.

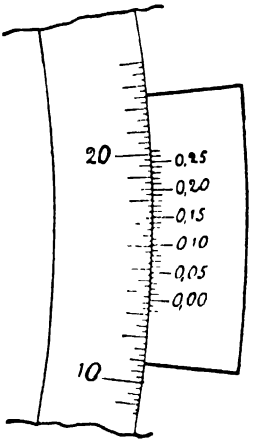


FIG. 16.—Polarimeter Vernier.

The reading indicated in the diagram of the scale and vernier shown in Fig. 16 is 13·5 + 0·16 = 13·66°.

If the reading is negative, i.e. if the zero of the vernier is below that of the scale, count the number of degrees which lie between the zero of the scale and that on the vernier. Suppose that this is between 3° and 4°, and that the zero of the vernier comes between the second and third sub-divisions, the reading thus indicated would be between − 3·25 and − 3·5. Observe, as before, which graduation mark on the vernier is exactly coincident with one on the scale, and in this case add the vernier reading to the *higher* limit of the negative reading. For example, if the 15 mark on the vernier is exactly coincident with one of the scale graduations the reading will be − 3·5 + 0·15 = − 3·35°.

It should be noted that when the zero of the scale moves *downwards*, i.e. in a *clockwise* direction, the reading is *positive*,

and conversely that when the zero is moved *upwards*, i.e. in *contra-clockwise* direction the reading is *negative* (see p. 80). Further, it should be remembered that it is the zero on the *vernier* which is fixed, and that it is the *scale* which moves.

Verification of the Zero of the Polarimeter.

Before starting work with a polarimeter it is necessary to see that the instrument is in adjustment, and that the *zero of the scale* really corresponds with the position of the analyser in which uniform illumination of the two halves of the field is obtained, i.e. with the *zero of the instrument* (see p. 82).

To find the zero of the instrument proceed in the following manner: Place a small quantity of a mixture of sodium chloride and borax in the spoon of the Bunsen lamp, light the burner, and turn the spoon into the flame so that an intense yellow light is produced. Arrange the polarimeter with its axis in the direct line of the light from the flame, and with the end of the instrument at a distance of about 22 cm.* from the burner; so that on looking through the eye-piece a bright yellow field is seen. Fill the two-decimetre tube with distilled water, and place it in position in the polarimeter. Carefully focus the eye-piece by drawing out or pushing in its lens until the vertical line dividing the two halves of the field is sharply defined. It is easier to focus the field when the instrument is not exactly in the zero position. Turn the scale so that its zero is coincident with that of the vernier; look through the instrument and see whether the two halves of the field are equally illuminated. At first the field will probably appear uniformly dark, but as the eye grows more accustomed to the illumination it will be found that light is being transmitted, and unless the zero of the instrument is considerably out of adjustment, the intensity of the illumination in the two halves of the field will be about the same.

In looking down the instrument care must be taken to avoid pushing in the lens of the eye-piece and thereby altering the focus.

Now turn the scale through a small angle in either direction, and observe that one-half of the field becomes much brighter than the other, and that on moving the scale in the other

* The distance from the instrument at which the lamp should be placed is usually given for each instrument, and in the case of the particular instrument in use in this laboratory this is given as 22 cm

direction, the other half of the field becomes brightly illuminated; so that on rotating the scale through a small angle first in one direction and then in the other the bright half of the field appears to change over rapidly from one side to the other. The position of equal illumination lies somewhere between these two positions.

Turn the scale slowly back again towards the zero position, until the two halves of the field are uniformly illuminated. This is best judged by moving the dial through the *least possible angle* first in one direction and then in the other. For this purpose the fine adjustment should be used. Clamp the scale by screwing up the milled head *k*. Then turn the milled head *m* carefully in and out until a position is found, such that the least possible rotation in either direction produces a contrast in the illumination of the two halves of the field. This may be taken as the position of uniform illumination or the zero of the instrument (see p. 82). Examine the scale and see whether the zero of the scale is exactly coincident with that of the vernier; if this is not the case, carefully note and record the reading of the scale.

Now release the screw *k* and rotate the analyser through a small angle, so that it is no longer in the zero position. Observe the field, adjust the instrument again to the zero position, and again record the scale reading.

Several observations should be made in this manner, and with a little practice the recorded readings should differ only by small fractions of a degree. The average of these readings may be taken as the zero reading of the instrument.

Unless the instrument is considerably out of adjustment the observed zero will only differ slightly, if at all, from the zero indicated by the scale and vernier, i.e. a small positive or negative reading may be obtained. If such is the case the zero of the instrument need not be exactly adjusted to that of the scale, but the zero reading should be recorded, and any other readings taken with the polarimeter should be corrected accordingly (see below).

The method of adjusting the zero in cases where this may be necessary, and also the use of the pointer *h* (see Fig. 13), are explained on p. 97.

Correction of the Reading.—Where the instrument is not exactly in adjustment, any observed reading should be corrected by subtracting the zero reading from the observed reading, *due allowance being made in all cases for the sign of the readings.*

Thus if C = the zero reading and if R = the observed reading, the correct reading of the solution produced is given by—

R — C where R and C are both positive.
R — (— C) = R + C, where R is positive and C negative.
— R — (— C) = — (R — C), where both R and C are negative.
— R — C = — (R + C), where R is negative and C positive.

DETERMINATION OF SUCROSE BY MEANS OF THE POLARIMETER.

Preparation of the Cane Sugar Solution.

Weigh out accurately 25 gms. of finely powdered lump or white granulated sugar*; dissolve the sugar in distilled water and dilute the volume to 250 c.c. For working with the polarimeter the solution must be perfectly clear and transparent, and, if necessary, it may be filtered rapidly through a dry filter paper.

Measurement of the Rotation Produced by the Sugar Solution—Direct Method.

Rinse out the 2-decimetre tube twice with small quantities of the solution, and then fill the tube with the solution (see p. 87). Wipe the ends of the tube and place it in position in the polarimeter.

Rotate the analyser slowly in a clockwise direction until a position is found where the bright half of the field changes over rapidly from one side to the other.† Then turn the analyser through a very small angle, first in one direction and then in the other, until the position of uniform illumination is found.

Observe and record the scale reading; turn the analyser slightly, again adjust to the position of uniform illumination, and again record the reading.

* White sugar should be used in preference to any of the brown forms, since the latter will give a slightly coloured solution and will therefore be more difficult to work with in the polarimeter.

† If difficulty is experienced in finding this position, the analyser has probably been rotated through too great an angle. Until more experience in using the instrument has been gained this difficulty may be obviated, in cases where the approximate composition of the sugar is known, by calculating approximately the rotation the solution is likely to produce; the scale can then be turned to this position and the position of uniform illumination may be readily found by rotating the analyser through a very small angle, first in one direction and then in the other.

The two readings should not differ by more than about 0·05°.

The whole operation should be repeated two or three times, and the average of the readings may then be taken as giving a correct measure of the rotation of the plane of polarised light produced by the sugar solution.

From this rotation the percentage of sucrose in the cane sugar may be calculated in the manner already indicated (see p. 83).

The following example will serve to show the method of working :—

Example.—A 10 per cent. solution of cane sugar gave a rotation of + 12·73° when measured in a 2-decimetre tube, the zero reading of the instrument being − 0·12°.

The corrected rotation of the solution =

$$12{\cdot}73 - (-\,0{\cdot}12^\circ) = 12{\cdot}73 + 0{\cdot}12 = 12{\cdot}85^\circ.$$

Then, since the specific rotatory power of sucrose = + 66·5°,

100 gms. of sucrose in 100 c.c. of water give a rotation of + 66·5° in a 1 dm. tube.

1 gm. of sucrose in 100 c.c. of water give a rotation of 0·665° in a 1 dm. tube.

1 gm. of sucrose in 100 c.c. of water give a rotation of 1·33° in a 2 dm. tube.

So that each gram of sucrose present in 100 c.c. of solution will give a rotation of 1·33° if measured in a 2-decimetre tube.

The observed rotation = 12·85°.

∴ The number of grams of sucrose present in

$$100 \text{ c.c. of the solution} = \frac{12{\cdot}85}{1{\cdot}33} = 9{\cdot}66.$$

Hence

10 gms. of the cane sugar used contain 9·66 gms. of sucrose.

or 100 „ „ „ „ 96·6 „ „

This result can also be obtained by substituting 66·5 for $[\alpha]_D$ in the expression given on p. 84.

Polarimetric Examination of an Inverted Sugar Solution—Method of Double Polarisation.

The percentage of sucrose present in the cane sugar should also be obtained from the change in rotation observed on

inversion, since this method is frequently employed for the determination of sucrose in mixtures containing sucrose and dextrose or sucrose and invert sugar (see p. 112).

Measure out 100 c.c. of the solution into a flask graduated for 100 c.c. and also for 110 c.c. Add 10 c.c. of concentrated hydrochloric acid, and warm the flask in a water bath heated to a temperature of about 85° C. until the contents of the flask reach 68°-70° C.; keep the flask at this temperature for five minutes,* and then cool by immersion in cold water. The total time of heating should be about ten minutes. After cooling, make up the volume to exactly 110 c.c. and note the temperature of the solution (see p. 95).

The solution has thus been diluted to $\frac{11}{10}$ of its original volume, but if the depth of solution used for the polarimetric determination is increased to $\frac{11}{10}$ of that originally observed (i.e. of that used before inversion), this dilution will be allowed for without any further correction being necessary.

For this purpose a 2·2-decimetre tube (= $\frac{11}{10}$ of 2-decimetre tube) is provided. Fill the 2·2-decimetre tube with the invert sugar solution; place the tube in the polarimeter and observe the rotation produced.

The rotation will in this case be negative, and if the scale is in the zero position it should be moved in a contra-clockwise direction in order to find the position of uniform illumination.

As before several observations should be made, and the averege of the readings taken.

If a 2·2-decimetre tube is not available, the observations may be made in a 2-decimetre tube, and the reading obtained multiplied by 1·1 to compensate for the dilution of the solution.

Taking the specific rotatory power of invert sugar $[\alpha]_D^{15°}$ to be $-$ 22·7°, it follows that a solution of sucrose which before inversion gave a rotation of + 66·5° will after inversion, give a rotation of $-$ 22·7° (at 15° C.). Thus the effect of inversion is to change the rotation from + 66·5° to $-$ 22·7°, that is, the angle turned through on inversion

$$= + 66·5° - (- 22·7°) = 89·2°.$$

It is thus possible to determine the amount of sucrose from the *change* in rotation produced on inversion. It is necessary,

* With more prolonged heating incipient decomposition is likely to occur (see p. 110), and the solution becomes yellow in colour; thus rendering obser vations with the polarimeter more difficult.

however, first to consider the effect of temperature changes on the rotation produced by sugar solutions.

The Effect of Temperature on the Rotation of Lævulose.—The specific rotatory power of lævulose decreases by 0·6385° for each degree rise in temperature.

Owing to this variation in rotation, the change in rotation produced on inverting a solution of sucrose (1 gm. in 1 c.c. measured in a 1-decimetre tube) will diminish by about $\frac{1}{3}$* of an angular degree for each degree rise in temperature. At 0° C. the angle turned through on inversion = 94·2°, and the change on inversion at a temperature of t° C. will be equal to $94{\cdot}2° - \frac{t°}{3}$. At 15° C. this will give $(94{\cdot}2 - 5)° = 89{\cdot}2°$, which is the value given above. Hence in making determinations with invert sugar solutions, the change in rotation, on inversion, produced by 1 gm. of sucrose dissolved in 1 c.c. of solution and measured in a 1-decimetre tube may be taken as $94{\cdot}2 - \frac{t}{3}$, where t = temperature in degrees centigrade. It should also be noted that since the specific rotatory power of lævulose decreases by 0·6385° for each degree rise in temperature, and $[\alpha]_D$ at 15° C. = −98·0, the specific rotatory power at 87° C. will be equal to

$$\begin{aligned} &- \{98{\cdot}0 - (87 - 15) \times 0{\cdot}6385\} \\ &= - 52{\cdot}1°. \end{aligned}$$

Hence at this temperature the specific rotatory power of lævulose is approximately equal in magnitude but opposite in sign to that of dextrose. Thus, at a temperature of about 87° C., a solution of invert sugar will have a zero rotation.

This fact is made use of in certain sugar determinations, the observations being made with a tube fitted with a hot water jacket and thermometer.

The following example will serve to show the method of calculating the percentage of sucrose from the change in rotation produced on inversion.

Example.—A 10 per cent. solution of cane sugar gave a rotation of + 12·81° before inversion and a rotation of

* One half of 0·6385; as in invert sugar lævulose is associated with an equal amount of dextrose. The rotation of the latter is not appreciably altered by changes of temperature.

$-4{\cdot}27°$ after inversion. The observations were made in a 2-decimetre tube at a temperature of 18° C.

The angle turned through on inversion

$$= 12{\cdot}81 - (-4{\cdot}27) = 17{\cdot}08°.$$

The change on inversion produced by 100 gms. of sucrose in 100 c.c. in 1-decimetre tube at a temperature of 18°

$$= 94{\cdot}2 - \frac{18}{3} = 88{\cdot}2°.$$

Hence 1 gm. sucrose in 100 c.c. in 1-dm. tube would give a change of $0{\cdot}882°$

" " " 100 c.c. " 2 " " $1{\cdot}764°$

So that for each gram of sucrose present in 100 c.c. of the solution there will be a change on inversion of $1{\cdot}76°$.

The observed change $= 17{\cdot}08°$.

$\therefore$ number of grams of sucrose in 100 c.c. of the solution

$$= \frac{17{\cdot}08}{1{\cdot}76} = 9{\cdot}70.$$

$\therefore$ 10 gms. of the cane sugar contain 9·7 gms. of sucrose.

or 100 " " " " 97·0 " "

Thus the cane sugar contains 97·0 per cent. of sucrose.

This result can also be obtained by substituting $88{\cdot}2$ $\left(94{\cdot}2 - \frac{t}{3}\right)$ for $[\alpha]_D$ in the expression given on page 84.

Other Sugar Solutions.

Dextrose and Lævulose.—Freshly prepared solutions of these sugars give a greater rotation than solutions which have been kept for some hours before examination.

This phenomenon of *mutarotation* is explained by the existence of two stereoisomerides of the sugar, one of high and the other of low rotatory power, a constant rotation being obtained when the two forms are in equilibrium with one another. A constant rotation can be obtained by allowing the solution to stand for some hours, by bringing the solution to the boil, or by adding a few drops of ammonia solution; the change being greatly accelerated by heat or by the action of acids and alkalies.

On this account solutions of dextrose or lævulose which are to be examined polarimetrically should either be allowed

to stand for some hours before making the observations or should be treated by one or other of the methods mentioned above. The determination is then carried out in the manner described for the determination of sucrose by the direct method (p. 92); taking + 52·5° as the specific rotatory power of dextrose and − 98·0° as the specific rotatory power of lævulose (at 15° C.).

In the case of lævulose a correction must, if necessary, be made for the temperature (see p. 95).

Lactose in Milk.—The polarimetric determination of lactose in milk has already been described (see p. 16).

Clarification of Sugar Solutions.

Sugar solutions which are to be examined polarimetrically, and which are not absolutely clear, or are slightly coloured, may be clarified by the addition of basic lead acetate solution, or by the addition of alumina cream (see below).

The least possible quantity of the clarifier should be used, and the reagent should be added drop by drop until a precipitate begins to separate out, leaving a clear solution above.

The solution is then made up to a definite volume in a graduated flask, mixed by shaking, and filtered through a dry filter. The first few cubic centimetres should be rejected and the remainder used for examination in the polarimeter.

(1) *Basic lead acetate* solution is prepared by heating 430 gms. of normal lead acetate, 130 gms. of litharge and 1000 cc. of water to boiling for half an hour. The mixture is allowed to cool and settle, and the supernatant liquid is then diluted to a specific gravity of 1·25 with recently boiled distilled water. Solid basic lead acetate may be substituted for the normal salt and litharge in the preparation of the solution.

(2) *Alumina Cream.*—A cold saturated solution of alum is divided into two unequal portions. A slight excess of ammonia is added to the larger portion and the remaining alum solution is added by degrees until a faintly acid action is obtained.

Note on the Adjustment of the Zero of the Polarimeter.

To adjust the zero of the polarimeter, turn the scale until its zero is exactly coincident with that of the vernier, and fix the scale in this position by turning the milled head *k*, Fig. 13.

and the subsequent determination of reducing sugars thus produced either by Fehling's solution or by the polarimeter. The equation

$$\underset{n \times 162}{(C_6H_{10}O_5)_n} + \underset{n \times 18}{nH_2O} = \underset{n \times 180}{nC_6H_{12}O_6}$$

shows that 162 parts of starch should give 180 parts of dextrose, or 100 parts of dextrose = 90 parts of starch, and the amount of starch is usually calculated from the dextrose produced from it on this basis.

Microscopical Examination of the Starches.

The microscope is used extensively in food analysis for the detection and identification of starch grains. Under the microscope starch is seen to be composed of well-defined granules, but the size and shape of the granules differ considerably for different kinds of starch.

The following starches should be examined under the microscope and their appearance noted: Wheat, Maize (Corn), Rice, Potato, and Arrowroot.

Place a minute quantity of the starch on a slide, add a drop of distilled water or of a mixture of water and glycerine. Cover with a slip, and wipe off any superfluous liquid; the specimen is then ready for examination. The same magnification must be used in each case, since the size of the granules is of importance in distinguishing the different kinds of starch: $\frac{1}{6}$-inch objective is convenient. The most important points to be observed in the microscopical examination of starches are: (*a*) the size and shape of the granule; (*b*) the position of the hilum; (*c*) concentric markings; (*d*) the appearance under polarised light.

Wheat Starch (Plate II. *A*).—The granules are rounded in shape and show concentric rings and hilum. The granules are chiefly of two sizes, large and very small. The larger granules show a faint cross-marking under polarised light.

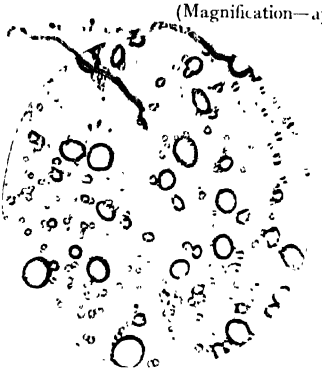
Maize (Corn) Starch (Plate II. *B*).—The granules are polygonal in shape, sometimes with rounded angles. The hilum shows as a well-defined crack or star in the centre of the granule.

The granules show a distinct cross-marking under polarised light.

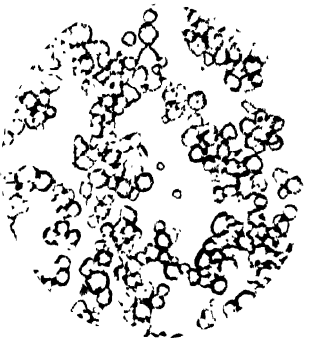
PLATE II

MICROPHOTOGRAPHS OF STARCHES

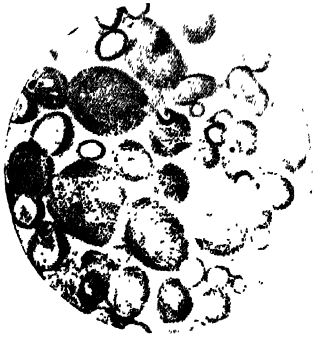
(Magnification—approximately 240)



A. Wheat

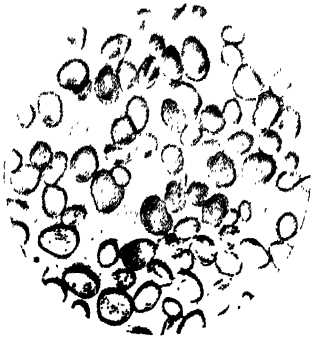


B Maize



C. Potato

D Potato (polarised light)



E. Arrowroot

F. Arrowroot (polarised light)

[*To face p.* 100

Rice Starch (Plate III. *A*).—Small pentagonal or hexagonal granules somewhat similar to maize, but smaller in size and with more sharply defined angles. The granules are too small to show a distinct cross-marking under polarised light, unless examined under a very high power.

Potato Starch (Plate II. *C*).—The larger granules are oval or oyster shape, and the smaller ones circular. The hilum shows as a spot usually towards the smaller end of the granule, and the larger granules show well-defined concentric rings. Under polarised light the granules show a very distinct dark cross usually towards the smaller end of the granule (Plate II. *D*).

Arrowroot Starch (Plate II. *E*).—The granules resemble those of potato, but on the average are smaller and more regular in shape. The hilum is usually towards the wider end, and the granules show faint but distinct concentric markings. Under polarised light a well-defined cross is formed towards the wider end (Plate II. *F*).

Examination of a Powder for Starch.

In examining a powder for starch, a few grains of the finely divided powder should be moistened with a drop of a very dilute solution of iodine in potassium iodide, instead of with water, and then examined under the microscope. The starch granules will be stained blue, and may be identified by their size, shape, etc., in the manner already described. If necessary, the starch may be separated from the soluble constituents of the powder by extraction with *cold* water. The insoluble starch is filtered off and examined under the microscope. (See examination of starch in baking powders, p. 196.)

Glycogen or Animal Starch.

Glycogen is a carbohydrate which is closely allied to starch, and since it is found principally in animal organisms it is sometimes called animal starch. It is, however, also found in certain vegetable organisms, notably yeast. Glycogen is a white amorphous powder which forms an opalescent solution with warm water. It gives a red-brown coloration with iodine, and is converted by diastase and by dilute acids into the same substances as are obtained from starch.

Dextrin.

Various forms of dextrin are obtained as intermediate products between starch and maltose, when the former is hydrolysed by dilute acids. Dextrin is soluble in water, but insoluble in alcohol, and does not reduce Fehling's solution. These properties serve to distinguish it from starch on the one hand, and reducing sugars, e.g. maltose and dextrose, on the other. It is usual to distinguish between two different forms of dextrin—*erythro-dextrin*, which gives a reddish-brown colour with iodine, and *achro-dextrin*, which gives no colour with iodine; both forms are insoluble in alcohol.

Dextrin has a high specific rotatory power, $[\alpha]_D = 200°$, and this fact should be borne in mind in making polarimetric observations on solutions of sugar products which may possibly contain dextrin.

Dextrin is prepared commercially by heating starch moistened with dilute nitric or hydrochloric acids to a temperature of 100° to 150° C., and is sold under the name of *British Gum*. It may also be prepared by heating dry starch to 200° C.

Dextrin also forms one of the constituents of glucose syrup (see p. 108), and this fact is made use of in testing honey, jam, etc., for adulteration with glucose syrup (see p. 116).

Determination of Dextrin.

Dextrin is sometimes determined polarimetrically, but it may also be separated quantitatively from a mixture containing reducing sugars by the addition of a considerable bulk of alcohol. The precipitated dextrin is separated by filtration through a weighed filter paper, washed thoroughly with alcohol, dried at 100° C., and weighed.

To demonstrate the properties of dextrin dissolve a small quantity of pure dextrin in water, and test portions of the solution in the following manner:—

(1) Add Fehling's solution and boil; if the dextrin is pure there will be no reduction.

(2) Add to the solution three or four times its own volume of alcohol; the dextrin separates out as a white precipitate.

(3) Add a drop of a solution of iodine in potassium iodide; a red-brown coloration shows the presence of erythro-dextrin. In examining a solution for small quantities of erythro-dextrin

this test may be carried out in the following manner: Measure out in two test tubes approximately equal volumes of a very dilute solution of iodine in potassium iodide. To one add a few cubic centimetres of the solution to be tested, and to the other add an equal volume of distilled water. On comparing the colour of the two solutions, any brown colour produced by the dextrin will be readily observed.

Commercial dextrin usually gives a slight reduction with Fehling's solution, this being due to the presence of a small amount of dextrose; whilst the colour obtained with iodine may be violet-brown instead of red-brown, owing to the presence of starch.

Cellulose.

The chemical reactions of cellulose have already been dealt with in Volume I. The framework of vegetable organisms is made up largely of cellulose and other related substances, e.g. ligno-cellulose (see Vol. I., pp. 87 and 126). Cellulose, with the exception of water, is found more abundantly in the vegetable world than any other substance. The *crude fibre*, as ordinarily determined in food analysis, may be defined as that portion of the food, other than mineral matter, which is insoluble in ether, and which resists the action of hot dilute acid and alkali. These reagents, ether, acid, and alkali, will remove fat, sugars, starch, and protein. The crude fibre is largely composed of cellulose.

Enzyme Action and Fermentation.

The changes which take place when the di- and poly-saccharides are heated with dilute solutions of acids or alkalis are due, as already stated, to the *hydrolysis* of these carbohydrates, i.e. to the interaction of the carbohydrate with water, and the acid or alkali added acts merely as a catalytic agent in increasing the rate of this hydrolysis.

In some cases the rate of hydrolysis can also be increased by the action of certain *organic catalysts* or *enzymes*. Enzymes are complex organic substances which are produced by living organisms, but the enzyme, once formed, can exercise its characteristic functions after it has been separated from the living organism, i.e. the presence of the living organism which produced it is not essential to the activity of the enzyme.

The exact nature and composition of these enzymes has not yet been established. It seems probable, however, that there is some intimate relation between the structure of the enzyme and that of the substance with which it reacts, since, unlike the inorganic catalysts, the enzymes are extremely specific in their action. Thus an enzyme may react with one sugar and be without action on the stereoisomeride of this sugar.

Enzymes behave as colloids, and as such do not form true solutions, but give rise to unstable systems with water which are extremely susceptible to outside influences.

In such a system the surface developed by the colloid enzyme will be relatively large, and the activity of the enzyme is probably mainly due to changes taking place at the interface of the enzyme and the substance with which it reacts.

The enzymes are usually most active at a temperature of 40° to 45° C. Most enzymes are destroyed if the temperature is raised above 60° C., whilst lowering the temperature has an inhibitory effect, and the rate of reaction is reduced. Some enzymes are most reactive in neutral solution, others in slightly acid or in slightly alkaline solution, and individual enzymes show other marked differences in properties.

The action of enzymes is not confined to hydrolysis, for some enzymes act catalytically in bringing about oxidising or other chemical reactions; but in all cases the enzyme may be regarded as acting as a colloidal catalyst which has distinctly specialised properties and limited activities.

Reactions of the Carbohydrates with Hydrolytic Enzymes.

Diastase.—Starch may be hydrolysed to dextrin and maltose by the enzyme *diastase*, which is secreted during germination by the embryo of certain plants, e.g. wheat and barley. The principal source of diastase is *malt extract.*

Malt and Malt Extract.—In the preparation of malt, barley grain is steeped in water for several days. The water is then poured off and the moist grain allowed to germinate. During germination the diastase content of the grain increases, and this is the chief object of "malting." When the maximum amount of diastase has been produced, as indicated by the length of the sprout grown by the grain, further germination is checked by drying. The depth of the colour of the liquid

obtained on extracting the malted grain with water depends largely on the temperature at which the grain is dried.

If dried between 32° and 38° C. it forms pale malt, whilst darker shades can be obtained by drying between 38° and 50° C.

The malt extract of commerce is prepared by extracting malted barley with water and concentrating the infusion, under reduced pressure, at a temperature low enough not to destroy the active properties of the diastase. Freshly prepared malt extract for laboratory purposes can be prepared in the following manner :—

Add 200 c.c. of water to 10 gms. of crushed malted barley, digest at the room temperature for two to three hours with occasional shaking, and then filter.

The method of using malt extract for hydrolysing starch has already been described (see p. 99). It should be remembered that when starch is hydrolysed by diastase the hydrolysis does not proceed further than maltose, whereas when acid is used the final product is dextrose.

Invertase and Maltase.—Sucrose can be converted to invert sugar by the enzyme *invertase*, and maltose to dextrose by the enzyme *maltase;* both these enzymes are present in yeast (see below).

The former, in accordance with the usual nomenclature, should more correctly be termed *sucrase*, since the name of the enzyme should indicate the substance with which it reacts, and not that of the product formed.

Alcoholic Fermentation.

Yeast is a fungus of the genus *Saccharomyces* which is found widely distributed in the vegetable kingdom, and also in the air. Yeast, in addition to containing the two enzymes mentioned above, contains the enzyme "zymase." This latter enzyme has the power of decomposing the monosaccharides with the formation of alcohol and carbon dioxide.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2.$$

For a long time the view was held that this conversion of dextrose to carbon dioxide and alcohol, usually described as *alcoholic fermentation*, could only be brought about by living yeast cells, since aqueous extracts of yeast which have been freed from yeast cells will not ferment sugars.

The work of Buchner, however, showed that the enzyme zymase is secreted within the yeast cell. So that if the *yeast cells are first broken down* by grinding with sand and kieselguhr, and the mass thus obtained extracted with water and carefully filtered until free from yeast cells, an extract is obtained which has the power of converting sugars to alcohol and carbon dioxide, i.e. of bringing about alcoholic fermentation. Although the term *fermentation* was first applied in connection with alcoholic fermentation, it is now used as a general term applied to a group of chemical changes which are consequent on the life and development of certain micro-organisms, e.g. souring of milk, formation of acetic acid from alcohol as in the production of vinegar, etc. When the term "fermentation" is employed without qualification, alcoholic fermentation is usually understood. It is necessary to point out that although *living* yeast is generally used to bring about the fermentation of sugar, this fermentation is actually effected by the *enzyme* zymase, which is secreted by the yeast cell within itself. It should also be noted that many by-products are obtained in fermentation reactions.

The Action of Yeast on Sugars.

The monosaccharides are directly fermentable by yeast, owing to the action of the zymase on the sugar.

Yeast, in addition to containing zymase, also contains the enzymes invertase and maltase, which, as already stated, will bring about the hydrolysis of sucrose and maltose respectively :-

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2C_6H_{12}O_6.$$

The monosaccharides thus formed will react with the zymase, so that both sucrose and maltose are fermentable by yeast.

Lactose is not fermented by yeast, since there is no enzyme present to bring about the hydrolysis of the lactose with the consequent formation of monosaccharides.

Yeast has no action on *starch*, since diastase is not present, but when mixed with *flour* fermentative changes occur.

These changes are to be accounted for by the presence of diastase in the flour, and also of small quantities of the products of hydrolysis of starch, e.g. maltose and dextrose.

(See sections on Flour, p. 119, and Bread-making, p. 244,

also sections on Vinegar, p. 171, and Alcoholic Beverages, p. 196.)

For further information on the subject of enzymes see "Enzyme Action," by Bayliss (Longmans), and "Alcoholic Fermentation," by Harden (Longmans).

SUGAR PRODUCTS.

A brief description of the nature and composition of some of the principal sugar products may now be given.

Cane Sugar.

Sugar is extracted from the sugar cane by crushing the cane in rolling mills and expressing the juice.

The juice is freed from nitrogenous matter, organic acids, etc., by heating to coagulate the proteins, and by the addition of lime to neutralise the acids. The inversion of the sugar during the subsequent processes is thus prevented. The insoluble matter which separates on heating is removed as a scum.

Any excess of lime will combine with the sugar. The compound thus formed, can, however, be decomposed and the sugar recovered by passing carbon dioxide through the mixture.

The solution is evaporated and concentrated under reduced pressure until the sugar crystallises. The crystals are separated from the mother liquor by draining or by means of a centrifuge.

The crystals thus obtained are yellowish in colour, but in the case of a good grade sugar may be sold without further refining as *Raw Sugar*, *Muscavado Sugar*, or *Brown Sugar*.

The mother liquor left after the separation of the crystals is known as *Molasses*, and may be refined and sold as *Treacle*, the coarser grades being used for the production of rum. In order to obtain white sugar the brown crystals of raw sugar are re-dissolved in water, and the solution is clarified and filtered through animal charcoal.

The solution is next concentrated as before until crystallisation takes place. In this manner colourless crystals of *white granulated sugar* are obtained, and the mother liquor from which these crystals separate is known as *drip syrup* or *golden syrup*.

A good grade of raw Muscavado or brown sugar should contain from 87 to 91 per cent. of sucrose, whilst white granulated sugar is composed almost entirely of sucrose, and may contain up to 99·8 per cent. of sucrose.

Beet sugar, which is also sucrose, is extracted from beet by a process similar to that employed for the extraction of cane sugar. The molasses obtained from sugar beet are, however, owing to the character of the nitrogenous compounds they contain, unfit for food.

Molasses, Treacle, and Golden Syrup.

The method of obtaining these products has been described above. They vary considerably in composition, but consist essentially of mixtures of sucrose and invert sugar with water, and differ chiefly in the nature and quantity of the constituents other than sugar which they contain. Golden syrup, which is the most refined form, only contains a very small proportion of such constituents, but it has no very typical composition. The analysis of an average sample is given below :—

Sucrose	= 34·40 per cent.
Dextrose and lævulose . . .	= 46·35 ,,
Water	= 18·5 ,,
Mineral matter and other organic matter	= 0·75 ,,

The methods employed for the analysis of golden syrup are dealt with on p. 111.

Commercial Glucose, Glucose Syrup, Starch Sugar, Corn Syrup.

The product sold under the above names is prepared by hydrolysing starch (frequently corn or maize starch) with dilute sulphuric acid. As soon as hydrolysis is complete, i.e. the mixture no longer gives a blue colour with iodine, the excess of acid is neutralised by the addition of finely-powdered chalk. The mixture is allowed to stand until the calcium sulphate has settled to the bottom of the vessel; the clear solution is then separated by decantation and concentrated to a syrupy consistency.

The chief constituents of this product are dextrose, maltose, and dextrin, but the relative proportions of these con-

stituents vary considerably in different samples, and dextrose is not necessarily present in excess of maltose and dextrin, hence the term "glucose syrup" is somewhat misleading. The composition of commercial glucose syrup, as stated by different observers, shows considerable variation. Leach * gives the following average composition :—

Dextrin	= 29 to 45·3 per cent.
Maltose	= 4·6 to 19·3 ,,
Dextrose	= 34·3 to 36·5 ,,
Ash	= 0·32 to 0·52 ,,
Water	= 14·2 to 17·2 ,,

Jago,* as the result of the analysis of four different samples, gives the following composition :—

Dextrin	= 16·2 to 21·4 per cent.
Maltose	= 49 to 60·9 ,,
Dextrose	= 7·5 to 14·26 ,,
Ash	= 0·18 to 0·26 ,,
Water	= 15·2 to 18·2 ,,

The examination of glucose syrup is dealt with on p. 111.

Honey.

Honey is a sugar product gathered and stored by bees. By means of an inverting enzyme secreted by the bee, the sucrose collected from the flowers is converted into invert sugar, and, chemically, honey consists essentially of a concentrated solution of dextrose and lævulose.

In some cases small amounts of sucrose may also be present, the other constituents being small proportions of mineral and flavouring matters, wax, pollen, etc.

A genuine honey should not contain more than 8 per cent. of sucrose, 0·25 per cent. of ash and 25 per cent. of water, whilst the reducing sugars usually amount to from 70 to 80 per cent. If the honey is unadulterated the dextrose and lævulose will be present in about equal proportions, and the honey will therefore be lævo-rotatory. In the case of some American honeys which are derived partly from *honeydew*, exudations produced on the leaves of certain plants, a honey of somewhat different composition is obtained, and such honey may give a positive rotation.

* See list of books of reference, p 268.

The chief adulterants of honey are glucose syrup and artificial honey, or artificial invert sugar.

Artificial Honey or Artificial Invert Sugar.

An invert sugar syrup similar in composition to honey can be obtained by heating a mixture of cane sugar and water with a small proportion of tartaric or citric acid.

Such a syrup may be conveniently prepared in the laboratory by Herzfeld's method: 1000 gms. of sugar, 300 c.c. of water, and 1·1 gm. of tartaric acid are heated to boiling-point for thirty to forty-five minutes. Under these conditions a straw-coloured syrup is obtained, with a taste not unlike that of honey, but somewhat lacking in flavour. The syrup is almost identical in composition with honey, but unless other substances are added will be deficient in ash. The most satisfactory tests for the presence of such syrups in honey depend on the formation of traces of oxymethylfurfural, a decomposition product of lævulose formed when sucrose is heated for some time with small amounts of acid. This substance may be detected by means of certain colour reactions (see p. 118). Genuine honey which has been heated for some time will give similar reactions, but as heating impairs the colour and flavour of the honey, the commercial product is seldom treated in this manner.

The methods employed for the examination of honey are dealt with on p. 116.

THE CHEMICAL EXAMINATION OF SUGAR PRODUCTS.

It is impossible within the scope of this course to describe in detail the various methods employed in the analysis of the various sugar products; the following practical work will, however, serve to indicate the general lines on which such analysis is carried out, and will afford illustrative examples of some of the more important methods.

Cane Sugar.

The methods of determining the percentage of sucrose present in a sample of cane sugar have already been dealt with (see pp. 75 and 92).

The methods adopted for the determination of this sugar in the presence of other sugars are dealt with under golden syrup, p. 111.

Reactions of Glucose Syrup.

Since glucose syrup is used as an adulterant of other sugar products, its properties and reactions should first be studied.

Dilute some of the syrup with an equal part of water and examine portions of the solution in the following manner :—

I. *Dextrose and Maltose.*—Prepare an osazone and try to identify glucosazone and maltosazone (see p. 70).

II. *Dextrin.*—(*a*) Add alcohol to precipitate the dextrin (see p. 102); (*b*) test for the presence of erythro-dextrin by the iodine reaction (see p. 102).

III. *Calcium Sulphate.*—The examination for calcium sulphate is usually made on the ash of the syrup (see method of examining ash of honey). In most cases, however, its presence may be detected in the aqueous solution.

(*a*) Add barium chloride and hydrochloric acid; barium sulphate is precipitated.

(*b*) To precipitate the calcium add ammonium hydroxide and ammonium oxalate; the precipitate obtained, if due to the formation of calcium oxalate, will be insoluble in acetic acid and soluble in hydrochloric acid.

Examination for Arsenic in Glucose Syrup.—The sulphuric acid used in the production of glucose syrup sometimes contains small quantities of arsenic and traces of arsenic may by this means be introduced into the syrup.

Arsenic is readily detected when present by the Gutzeit test, described on page 219. About 5 gms. of the sample should be used for the test.

Analysis of Golden Syrup.

As already stated, golden syrup is composed essentially of cane sugar, invert sugar and water, so that a determination of the amounts of sucrose, dextrose and lævulose present in a sample of this product will afford a convenient illustration of the methods employed in the analysis of mixtures of the sugars.

In order to obtain the proportions of the different sugars present in a syrup such as golden syrup, the following data are required :—

(1) The rotation produced by a given length of a 10 per cent. solution before inversion.

(2) The rotation produced by a given length of a 10 per cent. solution after inversion.

(3) The number of grams of reducing sugar (calculated as dextrose) present in 100 c.c. of a 10 per cent. solution of the syrup, determined by Fehling's solution.

The method of procedure is as follows: Fill a small beaker (50 c.c.) about two-thirds full with the syrup; weigh the beaker and syrup together with a short glass rod. Pour some of the syrup down the rod into a 250 c.c. graduated flask, weigh again, and in this way transfer about 25 gms. of syrup to the flask. The difference in the two weighings gives the weight of syrup taken for the experiment.

Add about 150 c.c. of water to the syrup in the flask, and if the solution is not quite clear or is rather dark in colour, clarify with basic lead acetate or alumina cream, dilute to 250 c.c., and filter through a dry filter (see p. 97). If the solution is clear and only slightly coloured, make up the volume without clarifying to 250 c.c.

(1) *Rotation Produced before Inversion.*—Allow the solution to stand for some hours, and observe the rotation produced at 15° C., using a 2-decimetre tube (see p. 92 and p. 96).

(2) *Rotation of the Inverted Solution.*—Invert 100 c.c. of the solution by the method described for cane sugar (see p. 94). Cool the solution to 15° C., and observe the rotation produced, using a 2·2-decimetre tube. If a 2·2-decimetre tube is not available use a 2-decimetre tube and multiply the reading obtained by 1·1 (see p. 94). Care should be taken not to overheat the solution, or it will darken in colour, and an accurate reading will be difficult to obtain.

(3) *Determination of Reducing Sugars by Fehling's Solution.*—Dilute 10 c.c. of the solution to 100 c.c. and find the number of cubic centimetres of this solution required to reduce 10 c.c. of Fehling's solution (see p. 75). Taking 10 c.c. of Fehling's solution as equivalent to 0·05 gm. of dextrose or lævulose, determine the number of grams of reducing sugar present in 100 c.c. of the *original* solution.

Determination of Sucrose.

The change in rotation on inversion will give a measure of the sucrose, since the dextrose and lævulose will not be appreciably affected by the process of inversion, if this operation is carefully carried out.

The number of grams of sucrose present in 100 c.c. of the solution may be calculated in the manner already described

for the determination of sucrose in cane sugar, by the method of double polarisation (see p. 93).

Thus for each gram of cane sugar present in 100 c.c. of the solution there will be a change on inversion of 1·78° if the rotation is measured on a length of 2 decimetres, at 15° C.

Hence the number of grams of sucrose present in 100 c.c. of the solution

$$= \frac{\text{Change on inversion}}{1{\cdot}78}.$$

If desired, the percentage of sucrose may also be found by making a determination with Fehling's solution of the amount of reducing sugar formed on inversion. For this purpose 20 c.c. of the 10 per cent. solution should be diluted to 100 c.c. and 50 c.c. of this diluted solution inverted and diluted to 100 c.c.,* in the manner described for cane sugar on page 75. The amount of reducing sugar found in this solution (calculated as dextrose), less the amount of reducing sugar found before inversion in the syrup solution of corresponding concentration, i.e. 10 per cent. solution diluted 1 in 10, will give a measure of the amount of sucrose present, on the basis that 0·05 gm. of dextrose is obtained from 0·0475 gm. of sucrose (see p. 74).

Determination of Dextrose and Lævulose.

The observed rotation of the solution before inversion is the algebraic sum of the rotations produced by the sucrose, dextrose and lævulose respectively. From the amount of sucrose present, obtained as described above, the rotation produced by the sucrose can be calculated, and this subtracted from the observed rotation will give the combined rotation of the dextrose and lævulose.

Thus since the specific rotatory power of sucrose = + 66·5°, 100 grams of sucrose in 100 c.c. would give a rotation

= + 66·5° in a 1-dm. tube.
1·0 ,, ,, ,, = + 0·665° ,, ,, ,,
or 1·0 ,, ,, ,, = + 1·33° ,, 2-dm. ,,

Hence the number of grams of sucrose present in 100 c.c.,

* In this way a one per cent solution of the syrup after inversion is obtained.

multiplied by 1·33° = rotation produced by sucrose. This result may also be obtained by using the expression given on page 84. Since $[\alpha]_D$ and (c) are known, the value of (a) can be calculated.

The rotation due to dextrose and lævulose = observed rotation minus rotation due to sucrose.

The total number of grams of reducing sugar (dextrose and lævulose) present in 100 c.c. of the solution is obtained from the determination with Fehling's solution, and from these two factors (i.e. the rotation and the copper-reducing power) the proportions of dextrose and lævulose may be calculated in the following manner :—

Let G = number of grams of dextrose present in 100 c.c. of the solution.

Then if R = total number of grams of reducing sugar present in 100 c.c. of the solution (determined by Fehling's solution), R − G = number of grams of lævulose present in 100 c.c. of the solution.

The specific rotatory power of dextrose = + 52·5°.
So that each gram of dextrose present in 100 c.c. will give a rotation = 0·525° if measured in a 1-decimetre tube, or 0·525° × 2 = 1·05° if measured in a 2-decimetre tube.

Therefore the rotation produced by the dextrose = G × 1·05°.

Similarly, since the specific rotatory power of lævulose = − 98·0° (at 15° C.),* the rotation produced by the lævulose = − 0·98° × 2(R − G) = − 1·96°(R − G).

Hence, if A = combined rotation of the dextrose and lævulose,

$$A = 1{\cdot}05^\circ \times G - 1{\cdot}96^\circ \times (R - G).$$

Thus, the values of A and R having been determined by experiment, the number of grams of dextrose (G) and the number of grams of lævulose (R − G) present in 100 c.c. may be calculated by means of the above expression.

The foregoing determinations give the number of grams of sucrose, dextrose, and lævulose present in 100 c.c. of the solution, and since this corresponds to 10 gms. of the syrup (assuming that exactly 25 gms. were diluted to 250 c.c.),

* If the observations are not made at 15° C. $[\alpha]_D$ for lævulose and also the change in rotation on inversion must be corrected for the temperature (see p. 95). For lævulose $[\alpha]_D^t = -\{98 - (t - 15) \times 0{\cdot}6385\}$.

these results, if multiplied by 10, will give the percentages of the sugars in the syrup.

The following numerical example will illustrate more fully the method of working.

Example.—A 10 per cent. solution of golden syrup gave a rotation of $+ 4{\cdot}02°$ before inversion and $- 3{\cdot}19°$ after inversion. A 2-decimetre tube was used before inversion and a 2·2-decimetre tube after inversion. The observations were made at 15° C. 10 c.c. of the 10 per cent. solution were diluted to 100 c.c. and 11·1 c.c. of the diluted solution were required to reduce 10 c.c. of Fehling's solution.

Determination of Sucrose.—The change in rotation on inversion

$$= 4{\cdot}02° - (- 3{\cdot}10°) = 7{\cdot}12°.$$

Each gram of sucrose present in 100 c.c. of the solution will give a change on inversion of 1·78° (see p. 94). Hence the number of grams of sucrose present in 100 c.c. $= \frac{7{\cdot}12}{1{\cdot}78} = 4{\cdot}0$ gms.

Rotation due to Dextrose and Lævulose.—The rotation produced by 4 gms. of sucrose dissolved in 100 c.c. of water, and measured in a 2-decimetre tube

$$= 4{\cdot}0° \times 1{\cdot}33° \quad \text{(see p. 113)}$$
$$- 5{\cdot}32°.$$

The rotation due to dextrose and lævulose

$$= 4{\cdot}02° - 5{\cdot}32°$$
$$= - 1{\cdot}30°.$$

Total Reducing Sugars.—The determination with Fehling's solution shows that 0·05 gm. of reducing sugar (dextrose and lævulose) are present in 11·1 c.c. of the diluted solution (see p. 73).

Therefore in 100 c.c. of the diluted solution (1 per cent.) there are $\frac{0{\cdot}05}{11{\cdot}1} \times 100$ gms. of reducing sugar. Or in 100 c.c. of the original solution (10 per cent.) there are $\frac{0{\cdot}05}{11{\cdot}1} \times 1000$ $= 4{\cdot}5$ gms. of reducing sugar.

Hence, by substituting— 1·30 for A and 4·5 for R in the expression obtained above (see p. 114), this gives—

$$\begin{aligned} -1{\cdot}30 &= 1{\cdot}05G - 1{\cdot}96(4{\cdot}5 - G) \\ -1{\cdot}30 + 8{\cdot}82 &= 3{\cdot}01G \\ 3{\cdot}01G &= 7{\cdot}5 \\ G &= 2{\cdot}5 \\ R - G - (4{\cdot}5 - 2{\cdot}5) &= 2{\cdot}0. \end{aligned}$$

The solution therefore contains 2·5 gms. of dextrose and 2·0 gms. of lævulose per 100 c.c.

Thus 10 gms.* of the syrup contain—

Sucrose = 4·0 gms.
Dextrose = 2·5 ,,
Lævulose = 2·0 ,,

Or the syrup contains—

Sucrose = 40 per cent.
Dextrose = 25 ,,
Lævulose = 20 ,,

Adulterated Syrups.

The results of an analysis carried out as described above will usually show whether commercial glucose has been added to the syrup.

If the syrup is unadulterated the dextrose and lævulose should be present in about equal proportions, and the rotation after inversion should be negative. If glucose syrup has been added, the dextrose will be considerably in excess of the lævulose. By such addition also an abnormally high rotation may be observed owing to the presence of dextrin, and the rotation after inversion may still be positive. The syrup should be tested for dextrin (compare honey, p. 117), since this substance will not be present in a genuine cane sugar syrup.

Examination of Honey.

Since honey is composed essentially of invert sugar, together with small amounts of sucrose, the method of

* That is, assuming that *exactly* 25 gms of the syrup were made up to 250 c.c. In any case the quantities of the different sugars found in 100 c.c. multiplied by $\frac{5}{2}$ will give the quantities present in the weight of syrup taken, and hence the percentages may be calculated.

analysis is similar to that employed for golden syrup, and need not therefore be described here. A sample of honey should, however, be examined for adulteration with glucose syrup, and also for adulteration with invert sugar (artificial honey), in the following manner:—

Glucose Syrup.—Genuine honey usually gives a small negative rotation when examined polarimetrically, but if glucose syrup has been added the rotation will almost certainly be positive (compare adulteration of golden syrup, p. 116). Some types of American honey are dextro-rotatory (see p. 109), so that a small positive rotation is not a conclusive proof of adulteration, though if a positive reading is obtained adulterants usually are present. Prepare a 10 to 20 per cent. solution of the honey, clarify if necessary with alumina cream, and add two or three drops of ammonia (see p. 96) before making up to the required volume. Examine the solution in the polarimeter and note if a negative reading is obtained.

Dextrin is not a constituent of honey, and its presence therefore indicates adulteration with glucose syrup.

Dilute the honey with an equal part of water, divide into two portions, and test in the following manner:—

(*a*) Gradually add alcohol, stirring constantly until a permanent turbidity is produced. In samples containing dextrin a white precipitate is formed which gradually settles, but with genuine honey only a slight turbidity is produced.

(*b*) Test for erythro-dextrin by the addition of iodine, as described under dextrin (see p. 103).

The percentage of mineral matter in honey is very small, and does not as a rule exceed 0·1 to 0·25 per cent. The addition of glucose syrup, which usually contains calcium sulphate, tends to increase the amount of mineral matter, so that evidence of adulteration may be obtained from a determination and examination of the ash, since calcium sulphate is not a constituent of unadulterated honey.

Weigh out from 5 to 10 gms. of the sample into a weighed platinum dish; evaporate to dryness on a water bath. Heat the residue cautiously over a low flame until frothing has ceased. Then increase the flame and ignite to a white ash at a low red heat. Cool in a desiccator and weigh.

If the weight of the ash exceeds 0·3 per cent. the honey has probably been adulterated.

Examination of the ash for calcium sulphate:—

Dissolve the ash in a few drops of hydrochloric acid, filter,

if not quite clear, through a small filter paper, and divide the solution into two portions. Test one portion for *sulphate* by the addition of barium chloride and the other for *calcium* by the addition of ammonia and ammonium oxalate solutions. The white precipitate obtained, if due to calcium oxalate, should be insoluble in acetic acid, but soluble in hydrochloric acid.

Invert Sugar (Artificial Honey).

The addition of invert sugar is usually detected by colorimetric tests, which are based on the presence of decomposition products of lævulose formed by heating invert sugar with acids during the process of manufacture.

In order to gain experience in carrying out these tests, a sample of artificial honey prepared as described on page 110 should be examined, and the colours produced compared with those obtained with the honey under examination.

Browne's Test.—The reagent, aniline acetate, is freshly prepared each time before use by shaking 5 c.c. of aniline with 5 c.c. of water and adding enough glacial acetic acid (2 c.c.) to clear the emulsion.

Dilute 5 c.c. of honey with 5 c.c. of water, and pour 1 to 2 c.c. of the reagent carefully down the sides of a test tube so as to form a layer upon the honey solution. If after gently shaking a red ring forms below the aniline layer and gradually spreads to the whole solution, invert sugar is present.

Fiehe's Test.—Ethereal extracts of invert sugar give a red coloration with a solution of resorcin in hydrochloric acid.

Dilute 5 c.c. of honey with 5 c.c. of water in a test tube and mix thoroughly. Add 5 c.c. of ether, shake the tube vigorously, and allow to stand until the ether layer is clear.

Transfer 2 c.c. of the clear ethereal solution to a clean test tube, add 1 to 2 drops of a hydrochloric acid solution of resorcin (1 part of resorcin in 100 parts of hydrochloric acid, specific gravity 1·19). Shake gently, and if a red colour develops, invert sugar is present.

Examination of the Ash.—In the case of a honey adulterated with artificial honey, the ash will probably be *low*, since the invert sugar contains no mineral matter (compare adulteration with glucose syrup, p. 117).

In the "Analyst" (Vol. **46**, 1921, p. 500) reference is made

to the specifications adopted for "artificial honey" by a conference of German chemists.

It was considered that the addition of some natural honey as flavouring agent, and of artificial colouring matter, should be allowable, and the addition of glucose syrup, if of good quality, up to a total amount of 20 per cent. permitted. The total solid matter should exceed 78 per cent. and the uninverted cane sugar be not more than 25 per cent.

The product must give a strong reaction with Fiehe's test, and be distinctly labelled "Artificial Honey."

The sweetening power of sugar and the changes which take place on heating sugar are dealt with in Chapter X.

FLOUR.

Although most vegetable foods contain a proportion of starch, the cereals may be regarded as the most important group of starch-containing foods.

Cereals are sometimes used as foods in the granular form, e.g. rice, pearl barley, etc., but more often in the form of a finely divided powder, known as "meal" or "flour," which has been obtained from the grain by milling or grinding. On account of its special bread-making properties, the flour of the wheat grain is used far more extensively than that of any other cereal, and unless otherwise qualified the term "flour" is understood to mean that of wheat.

Properties and Composition of Flour.—The grain of wheat consists of various parts. The true seed which is the *embryo* or *germ*, is that portion of the grain which ultimately develops into the plant. The main portion of the grain, however, which is composed principally of starch, is termed the *endosperm*, and its function is to supply the germ with food during the first stages of growth.

Besides the germ and the endosperm there are various outer and other coatings destined for the adequate protection of the seed, and these together form the *bran*.

Flours are usually graded according to the proportion of bran and germ included in the flour; the finest grades of "white flour" contain practically only the endosperm.

A "wholemeal flour" should, as the name implies, include all portions of the grain, whilst other flours are sometimes spoken of in terms of the percentage of the grain which is

included in the flour. Thus in an " 80 per cent.," also known as " standard " flour, 80 per cent. of the total constituents of the wheat grain should be included in the flour.

The germ of wheat, in addition to containing protein, also contains a high proportion of fat, and the inclusion of the germ in the flour is liable to impair its keeping properties, owing to this fat becoming rancid. The bran is largely composed of cellulose and proteins, and also contains the colouring matter of the grain. The inclusion of the bran thus gives a colour to the flour and increases the proportion of protein, but the bread obtained from wholemeal flour is usually considered to be less easily digestible than that made from white flour. Wheat flour, when moistened with water, forms a stiff tenacious dough or paste, and in this respect differs from the flour of other cereals. Oatmeal, for example, when similarly treated produces a damp mass having little or no tenacity. On kneading a mass of wheaten dough, enclosed in a piece of muslin, in running water until the starch has been washed away, there remains behind a greyish-white, sticky, elastic mass to which the name of *gluten* is given.

This substance consists of the insoluble proteins of the flour, together with small amounts of mineral matter, carbohydrates, and fat. Flours of other cereals, even if they are rich in protein, e.g. oatmeal, lack this power of forming gluten, and it is to this power that the special bread-making properties of wheat flour are to be attributed. In fact, it is on certain physical properties rather than on its purely chemical properties that the commercial value of a flour depends.

" Strength," colour, and flavour are the factors to which the baker attaches most importance.

Strength.—The " strength " of a flour is defined as its power to produce a large, bold, well-risen loaf, and this power is chiefly dependent on the physical characteristics, viz., viscosity, elasticity, and gas-retaining power, of the gluten of the flour.

Unfortunately there is at present no satisfactory method of numerically registering " strength," except by means of baking tests in which the volume of the loaf produced can be measured, and for this reason the strength of flours can be more suitably discussed in connection with bread-making * (see Chapter IX, p. 244).

*A series of papers by Gortner and Sharp on the physico chemical properties of strong and weak flours have recently been published in the " Journal of Physiological Chemistry " (1922 and 1923).

Colour.—The colours of different flours may be compared by making matching tests, and the baker will give preference to those which are lacking in colour, that is, a flour is considered to be a good colour when it is "white," or, more correctly, colourless. Flours are sometimes chemically bleached to improve their colour, i.e. to remove their colour, and methods of testing for bleached flour are described later (see p. 124).

Flavour.—Although the question of flavour is of practical importance, it is essentially a matter for the palate and for individual judgment. It cannot be tested for by chemical means.

Flours which are lacking in strength are sometimes superior in flavour to stronger flours, and good results can often be obtained by a suitable blending of flours.

Chemical Examination of Flour.

The chemical analysis of flour presents no special difficulties, and determinations of protein, fat, ash, etc., can be made by the usual methods employed in food analysis. As, however, no means of correlating the results of such an analysis with the "strength" of the flour has yet been devised, the examination may be conveniently confined to those tests and determinations which are likely to yield information of practical value. Some of the more important of these may be briefly discussed here.

Soluble Extract and Acidity.

Flour ordinarily contains certain constituents which are soluble in water, e.g. soluble starch, sugars, etc., but in addition to these, soluble substances may be formed, when the flour is extracted with water, by reactions, which are set up when the flour is moistened. For example, any broken starch grains present will be acted upon by the diastase of the flour forming maltose and dextrin. In the case of a good flour the proportion of solid matter extracted by cold water is relatively small, usually about 5 to 6 per cent. If, however, a large proportion of damaged starch grains is present and the flour is undergoing a process of degradation, the proportion of solid matter extracted, and probably also the acidity, will be considerably higher.

The percentage of solid matter in the cold water extract and the acidity may be determined in the following manner:

Mix 25 gms. of flour to a thin cream with water and transfer to a 250 c.c. graduated flask. Make up the volume with distilled water, which has been previously boiled to expel carbon dioxide and then cooled.

Cork the flask tightly and shake well, at intervals, for two to three hours, then allow the flask to stand until most of the insoluble portion has subsided.

Decant the liquid through a dry filter paper, and return the filtrate to the filter until a perfectly clear filtrate is obtained.

Soluble Extract.—Evaporate 50 c.c. of the filtrate to dryness in a weighed glass dish on a water bath, and dry the residue in the steam oven until a constant weight is obtained. This gives the weight of soluble matter obtained from 5 gms. of flour, so that the percentage is obtained by multiplying this result by 20.

The soluble matter may vary in the case of a normal flour from 3 to 7 per cent., but is usually between 5 and 6 per cent.

Acidity.—To determine the acidity, transfer 100 c.c. of the clear filtrate to a white porcelain dish, and titrate with decinormal sodium hydroxide solution, using phenolphthalein as indicator.

The acidity is usually returned as lactic acid (CH_3 . CHOH . COOH), 1 c.c. of decinormal sodium hydroxide being equivalent to 0·009 gm. of lactic acid.

In a good flour the acidity as determined by this method will usually not exceed 0·025 per cent. If the acidity is determined in the presence of the flour, i.e. by mixing the flour to a cream with water and titrating the mixture instead of on the filtered extract, higher values are obtained, but even in this case the acidity should not exceed 0·1 per cent.

Determination of Gluten.

Gluten, as already stated, is composed chiefly of the insoluble proteins of the flour, and is probably a loose compound of the two proteins gliadin and glutenin (see p. 157). A gluten determination does not give a measure of the total protein in the flour, and if this is required a Kjeldahl determination (using about 1 gm. of flour) should be carried out in the usual manner (see p. 11). The factor in this case for the calculation of the percentage of protein should be taken as 5·7. A gluten determination is to be regarded as an

estimation of the amount of those bodies, on the physical properties of which the elasticity and gas-retaining power of the dough depend.

It is usual to weigh the gluten both in the moist and in the dry condition, as in this way some idea of the water-absorbing capacity of the flour can be obtained. The percentage of gluten may be determined approximately by the following method: Mix 10 gms. of flour with 6 to 7 c.c. of water in a porcelain dish, and work the dough up into a ball, taking care that none of the flour is left adhering to the dish.

Tie up the dough securely in a piece of butter muslin, and knead gently under a stream of cold water from the tap until the water runs away clear, showing that all the starch has been washed out.

Place the ball of gluten thus formed in cold water for one hour, then press it to remove as much moisture as possible, transfer it to a weighed dish, and weigh. This will give the weight of *moist gluten*. To obtain the weight of *dry gluten* dry in a steam oven to a constant weight.

On the average about 35 per cent. of *moist* gluten will be found.

Mineral Matter in Flour. Chloroform Test.

Small quantities of certain inorganic salts, e.g. alum, phosphates, etc., are sometimes added to flour with a view to improving its colour or increasing the size of the loaf obtained from it.

These substances are known as "flour improvers," and their function is discussed in the section on bread-making, p. 247. If the ash of a flour exceeds 0·75 per cent. mineral salts have probably been added to the flour, but salts may be added in amounts which are too small to be detected with any certainty by ash determinations. The presence of small amounts of added mineral matter can, however, be shown by shaking up the flour with chloroform.

Chloroform has a higher density than the normal constituents of the flour, but a lower density than that of the added mineral salts, so that if the flour is shaken with chloroform and then allowed to stand, the flour will form a layer above the chloroform, and the salts will collect as a sediment at the bottom.

Fill a small separating funnel one-third full of flour, and

then add chloroform to within about 1 inch of the top of the funnel. Stopper or cork the funnel, shake vigorously, and then allow to stand for some hours until the chloroform has cleared, and note whether any sediment is left.

The method may be made quantitative by working with a known weight of flour. The sediment, together with a small portion of the chloroform, is withdrawn from the bottom of the funnel, and is then shaken up with a little fresh chloroform. After settling, the sediment is again separated from the bulk of the chloroform. The remainder of the chloroform is removed from the sediment by evaporation on a water bath, and the residue is then weighed.

Tests for Alum.—Special tests are employed for the detection of alum in flour and bread, but since the addition of this substance to any food is illegal in this country, it is now seldom found in flours. Tests for the detection of alum in flour and bread are described in "Food Inspection and Analysis," by Leach, and in other books on food analysis.

Bleached Flour.

It has already been pointed out that the baker attaches a good deal of importance to the question of colour, and aims at obtaining a flour which is as nearly colourless as possible. For this reason flours are sometimes bleached by chemical processes to improve (i.e. to remove) their colour. This bleaching is generally effected by treating with oxides of nitrogen, but ozone and chlorine are also sometimes used for this purpose.

Small quantities of nitrites left in the flour by the bleaching agent can be detected by the Griess-Ilosvay reaction. If bleached flour be treated with a solution of a naphthylamine salt and sulphanilic acid, an amino-azo dye of a red colour is at once produced (compare detection of nitrites in water, Vol. I., p. 13).

The Griess-Ilosvay Reagent is prepared in the following manner :—

No. 1.—0·5 gm. of sulphanilic acid is dissolved by heating in 150 c.c. of 20 per cent. solution of acetic acid.

No. 2.—0·2 gm. of alpha-naphthylamine hydrochloride is dissolved by heating in 150 c.c. of a 20 per cent. solution of acetic acid.

The two solutions are kept separate and mixed in equal proportions as required.

Testing of Flour for Nitrites.—20 gms. of flour are placed in a stoppered bottle with 200 c.c. of water and shaken at intervals for half an hour. The mixture is allowed to settle, and a portion filtered through a washed filter paper.

10 c.c. of the filtrate, to which 2 c.c. of the Griess-Ilosvay reagent and 50 c.c. of water have been added, are heated on a water bath at 80° C. for five minutes. In the absence of a pink coloration there are no nitrites present in the flour.

The test is extremely sensitive, and should be carried out in an atmosphere which is free from oxides of nitrogen, preferably in the open air.

The quantity of nitrite present may be determined by matching the colour obtained against that produced by a known volume of a standard nitrite solution.

If more than one part of nitrite (as nitrous acid) per million is found, the flour has probably been bleached (see below).

Bleaching by Chlorine.—If the flour has been bleached by the action of chlorine small amounts of chlorine will be absorbed by the fat in the flour. Chlorine in flour is usually detected by extracting the dried flour with ether or benzene, and examining the fatty residue obtained after the removal of the solvent.

The presence of chlorine in the residue may be shown by treating a small portion on a copper wire in a Bunsen flame, the wire having been previously heated until it no longer coloured the flame (see Vol. I., p. 173). A blue coloration indicates the presence of chlorine. It should be noted also that the absorption of chlorine will tend to reduce the iodine value of the fat, and if an abnormally low iodine value were obtained the presence of chlorine would be indicated.

Effect of Ageing on the Colour of Flour.—Flour on keeping becomes whiter in colour, and changes similar in character, but less marked in degree, to those observed in the case of bleached flour take place. Small amounts of nitrites, absorbed from the air, may be found in such a flour, but in general the amounts will be appreciably less than those found in bleached flour. If more than one part of nitrite (as nitrous acid) per million is present in the flour, the flour has probably been bleached, and with quantities above this the bleaching amounts to a practical certainty.

Chapter IV.

RAISING AGENTS.

The dough used in the preparation of bread, cake, etc., is aerated and rendered light and spongy by the production of carbon dioxide gas in the mixture. The carbon dioxide may be generated either by the action of yeast on sugar, *fermentation process;* for, or by the action of a chemical raising agent or *baking powder*, which is essentially a powder which gives off carbon dioxide when mixed with water and heated.

In this latter case the raising or leavening action is confined entirely to the liberation of carbon dioxide, and is unattended by the slight changes in the flour, decomposition of sugar and soluble proteins, which occur during the fermentation process. The fermentation process is dealt with in the section on bread-making (see p. 244).

COMPOSITION OF BAKING POWDERS.

Baking powders are usually prepared from the following constituents:—

(1) *A soluble carbonate*, generally sodium bicarbonate (or "baking soda"), but ammonium carbonate is also sometimes employed.

(2) *An acid or acid-reacting salt*, which acts on the carbonate, liberating carbon dioxide.

(3) *A dry inert substance*, which acts as a diluent of the active ingredients, and which tends to prevent their premature reaction.

For this purpose some form of starch (usually rice or maize) is almost universally employed; other substances occasionally used are "sugar of milk" (lactose) or dried milk powder.

Function of the Carbonate.

Although in the preparation of a baking powder sodium bicarbonate is usually mixed with an acid, it can be employed

as a raising agent by itself, since it gives off carbon dioxide on heating:

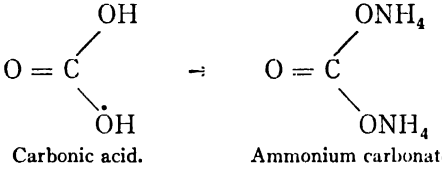
$$2NaHCO_3 \rightarrow Na_2CO_3 + H_2O + CO_2$$

2×84 gms. 44 gms.
84 ,, 22 ,,

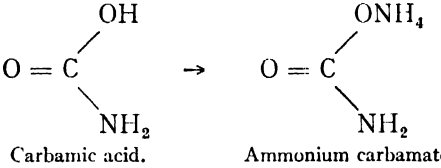
The alkaline residue of sodium carbonate, however, tends to produce a disagreeable flavour,* and this difficulty is obviated and double the yield of carbon dioxide obtained if an acid is mixed with the carbonate (see p. 128).

Ammonium carbonate is sometimes mixed with the sodium bicarbonate used for baking powders, but more frequently it is employed alone and without the addition of an acid.

Commercial ammonium carbonate is sold as a raising agent under the name of *volatile*, and consists of a mixture of ammonium carbonate and carbamate. The formulæ for these substances are shown below :—



Carbonic acid. Ammonium carbonate.



Carbamic acid. Ammonium carbamate.

On being heated, ammonium carbonate and carbamate both decompose, giving off carbon dioxide and ammonia—

$$(NH_4)_2CO_3 \rightarrow 2NH_3 + H_2O + CO_2$$

Ammonium carbonate.

$$NH_4CO_2 . NH_2 \rightarrow CO_2 + 2NH_3$$

Ammonium carbamate.

This substance is seldom found in made-up preparations, since the baking needs careful regulation to ensure the complete removal of the ammonia, so that the preparation may be free from all taste and smell of this gas.

* Sodium bicarbonate alone is generally used in mixtures containing some strong flavouring agent, e.g. gingerbread, so that the alkaline flavour is masked.

Function of the Acid.

Baking powders differ chiefly in the nature of the acid used. For this purpose an acid is required which is non-poisonous, soluble in water, solid under ordinary conditions, and which can be finely powdered and mixed with bicarbonate and starch to form a homogeneous mixture. The acid substances most frequently employed are the following:—

(1) Tartaric acid, $HOOC(CHOH)_2COOH$.

(2) Potassium acid tartrate—Cream of tartar,

$$HOOC(CHOH)_2 . COOK.$$

(3) Acid calcium phosphate, $CaH_4(PO_4)_2$.

(4) Potassium acid sulphate, $KHSO_4$.

(5) Alum, $K_2SO_4 . Al_2(SO_4) . 24H_2O$. Owing to hydrolysis this salt gives an acid reaction when dissolved in water.

Of these the first three are the most important. Potassium acid sulphate is sometimes used as a cheap substitute for tartaric acid, but the acid phosphate also answers this purpose, and is more frequently employed. The use of alum is illegal in this country (see p. 124), but not in other European countries or in the United States. The reactions of the above acids with sodium bicarbonate and also the relative proportions by weight in which they react are given in the equations below.

For the sake of convenience in working out the quantities required for making up a baking powder, the amount of acid required to neutralise 10 parts of sodium bicarbonate is also given in each case.

Tartaric Acid.—

$$2NaHCO_3 + HOOC(CHOH)_2COOH \rightarrow NaOOC(CHOH)_2COONa + 2CO_2 + 2H_2O$$

2×84 gms.	150 gms.	2×44 gms.
84 ,,	75 ,,	44 ,,
10 ,,	8·9 ,,	5·2 ,,

Cream of Tartar.—

$$NaHCO_3 + HOOC(CHOH)_2COOK \rightarrow NaOOC(CHOH)_2COOK + CO_2 + H_2O$$

84 gms.	188 gms.	44 gms.
10 ,,	22·4 ,,	5·2 ,,

Acid Calcium Phosphate.—

$$NaHCO_3 + CaH_4(PO_4)_2 \rightarrow NaCaH_3(PO_4)_2 + CO_2 + H_2O$$

84 gms.	233 gms.	Calcium monosodium trihydrogen phosphate.	44 gms.
10 ,,	27·7 ,,		5·2 ,,

The residue of calcium monosodium trihydrogen phosphate thus obtained is acid in reaction and also to taste, but if the proportion of bicarbonate is increased further replacement of the hydrogen in the acid phosphate takes place, thus :—

$$2NaHCO_3 + CaH_4(PO_4)_2 \rightarrow Na_2CaH_2(PO_4)_2 + 2CO_2 + 2H_2O$$

2 × 84 gms.	233 gms.	Calcium disodium dihydrogen phosphate.	2 × 44 gms.
84 ,,	116·5 ,,		44 ,,
10 ,,	13·9 ,,		5·2 ,,

In this case the residue, calcium disodium dihydrogen phosphate, is neutral in reaction, but slightly alkaline in taste. Thus, in order to obtain a residue which is neither acid nor alkaline to taste, the proportion of acid used should lie somewhere between the values indicated in the two equations given.

If the acid phosphate used is moderately pure and dry, a mixture of 10 parts of bicarbonate with 22·5 parts of the phosphate is found to give satisfactory results in practice. Much of the acid phosphate sold is, however, impure, and may contain considerable amounts of calcium sulphate (see p. 131), so that the proportion to be used can only be arrived at by experiment.

Potassium Acid Sulphate.—

$$CO_3 + KHSO_4 \rightarrow NaKSO_4 + CO_2 + H_2O$$

84 gms.	136 gms.	44 gms.
10 ,,	16·2 ,,	5·2 ,,

The Preparation of Baking Powder.

In preparing a baking powder it is essential that—

(1) All the ingredients should be perfectly dry, otherwise rapid deterioration takes place owing to the interaction of the carbonate and acid.

(2) All the ingredients should be reduced to a finely divided condition and mixed thoroughly, so that a homogeneous powder is obtained.

(3) The bicarbonate and acid should be mixed in such proportions that a nearly neutral residue is obtained. The

required proportions are readily calculated from the equations given above, but a slight excess of the bicarbonate is usually allowed for "covering," i.e. to cover any acid taste and to ensure that the residue is slightly alkaline rather than slightly acid.

Starch may be added in any proportion desired, and different brands of baking powder show considerable variation in this respect. It is clear that the amount of baking powder which should be added, per pound of flour, will depend on the extent to which the active ingredients have been diluted with starch, and in the case of commercial preparations it may vary from 1 to 4 teaspoonfuls (about 4 to 16 gms:) per pound of flour, according to the variety.

It is also important to note that, assuming sufficient acid is present to neutralise all the carbonate, the amount of carbon dioxide produced depends entirely on the quantity of sodium bicarbonate present, and is independent of the nature of the acid used.

Thus the above equations show that 84 parts of sodium bicarbonate yield 44 gms. of carbon dioxide (= 22·4 litres at N.T.P.) on neutralising with an acid, or 10 parts of bicarbonate yield 5·2 gms. of carbon dioxide $\left(= \frac{22{\cdot}4}{44} \times 5{\cdot}2 = 2{\cdot}65\right.$ litres at N.T.P.$\left.\right)$. If the sodium bicarbonate is heated alone, without the addition of an acid, only half this volume of gas is obtained (see equation, p. 127).

Since there is little difference in the cost of tartaric acid and cream of tartar, it is more economical to use tartaric acid, for, as the equations show (see p. 128), 8·9 parts by weight of tartaric acid are equivalent in neutralising power to 22·4 parts by weight of cream of tartar.

The special value of cream of tartar from the baker's point of view lies in the fact that it is less soluble than tartaric acid in cold water, and reacts slowly with the bicarbonate in the cold, though a vigorous action takes place on warming. Hence in a cream of tartar baking powder there is little loss of carbon dioxide on mixing, and a rapid evolution of gas on heating in the oven.

In order to combine economy with delayed evolution of carbon dioxide, baking powders are frequently prepared with a mixture of cream of tartar and tartaric acid.

Acid calcium phosphate provides a cheap substitute for

tartaric acid, but tends to take up moisture from the air, and when mixed with sodium bicarbonate and a relatively small proportion of starch the mixture deteriorates on keeping. For this reason the acid phosphate is not used very extensively in the preparation of baking powders, but is frequently employed in the production of "self-raising flour" (see p. 142), where the active ingredients are mixed with a large proportion of flour.

Acid calcium phosphate is prepared from bone-ash, which contains a large proportion of tricalcium phosphate, $Ca_3(PO_4)_2$. On treatment with phosphoric acid the following reaction takes place:—

$$Ca_3(PO_4)_2 + 4H_3PO_4 \rightarrow 3CaH_4(PO_4)_2.$$

In order to reduce the cost of production sulphuric acid is frequently mixed with, or substituted for, the phosphoric acid:

$$Ca_3(PO_4)_2 + 2H_2SO_4 \rightarrow CaH_4(PO_4)_2 + 2CaSO_4.$$

The acid phosphate prepared in this manner contains considerable amounts of calcium sulphate, and this latter substance is thus introduced as an impurity into preparations in which the acid phosphate is used as part of the raising agent. (For further information on this subject, see Local Government Report (1911), Food Reports, No. 13.)

The Use of Hydrochloric Acid.—Although hydrogen chloride cannot, on account of its gaseous nature, be utilised in made-up baking powders, raising may be effected by adding a solution of the acid to the dry materials (i.e. flour, etc.) with which the required proportion of sodium bicarbonate has previously been mixed:

$$NaHCO_3 + HCl \rightarrow NaCl + H_2O + CO_2$$

84 gms.	36·5 gms	44 gms.
10 „	„	5·2 „

Under these conditions sodium chloride or common salt is the only residue obtained from the reaction, and the admixture of other mineral salts, e.g. phosphates and tartrates with the food is avoided.

The process is sometimes used in the preparation of wholemeal bread, but is somewhat inconvenient in practice, since the volume of hydrochloric acid required must be carefully determined and measured out for each baking. Also, there

will be a rapid evolution of gas as soon as the ingredients are mixed.

EXAMINATION OF BAKING POWDERS.

Since the essential function of a baking powder is to liberate carbon dioxide when mixed with water and heated, the most important determination is the measurement of the amount of carbon dioxide given off from a known weight of the powder.

It is customary to measure both the *available* and the *total* carbon dioxide.

The *available carbon dioxide* is obtained by measuring the gas liberated from a known weight of powder on mixing with water and *heating*. It is therefore a measure of the amount of gas which is available for raising purposes. As already stated, sodium bicarbonate is usually present in slight excess of the acid (see p. 130). On heating, this excess of bicarbonate will be converted into sodium carbonate with the liberation of *half* its carbon dioxide (see p. 127), but when excess of acid is added to the mixture the carbonate is completely decomposed and the whole of the carbon dioxide liberated. The total carbon dioxide is therefore obtained by measuring the carbon dioxide liberated when a known weight of the powder is mixed with dilute acid.

The determination of the total carbon dioxide in a baking powder may be carried out in the manner already described for the determination of carbon dioxide in carbonates. (Vol. I., p. 41.)

For the determination of available carbon dioxide the same apparatus is used, but water must be substituted for acid in the side tube, and the flask must be carefully heated as soon as the reaction in the cold has ceased.

Determination of Total Carbon Dioxide by Volume, using the Nitrometer.*

Weigh out 0·2 to 0·4 gm. of the powder, and proceed as described in Vol. I., p. 42.

* Since in the determination of available carbon dioxide it is necessary to heat the mixture for some little time, it is not advisable to use the nitrometer for this determination, as on expansion the gas may drive the liquid out of the pressure tube.

Determination of Total and Available Carbon Dioxide by Weight.

Total Carbon Dioxide.—Use 1 gm. of the powder and proceed as described in Volume I., p. 41.

Available Carbon Dioxide.—Use 1 gm. of the powder, proceed as described for total carbon dioxide, but fill the side tube with water instead of dilute acid. When effervescence has ceased, the flask must be heated, but care is required to avoid charring the starch.

Heat the flask for a few seconds over a flame, allow to cool for a few seconds, and then heat again. Continue this alternate heating and cooling of the flask for about ten minutes, keeping the contents of the flask well shaken throughout the process.

After drawing air through to dispel the carbon dioxide, cool to the room temperature and weigh. From the loss in weight the percentage of available carbon dioxide is calculated.

Total Sodium Bicarbonate.

From the total carbon dioxide the percentage of sodium bicarbonate in the powder can readily be calculated. 44 gms. of carbon dioxide (or 22·4 litres at N.T.P.) are obtained from 84 gms. of sodium bicarbonate on complete neutralisation with an acid (see p. 128). It is often convenient for practical purposes to express the result as sodium bicarbonate instead of as carbon dioxide.

Excess of Sodium Bicarbonate.

The available carbon dioxide is made up of the carbon dioxide liberated by the interaction of the acid and bicarbonate in the powder, together with the carbon dioxide liberated by the conversion of the excess of sodium bicarbonate into carbonate on heating (see pp. 128 and 127). Assuming that, owing to the heating of the liquid, this latter reaction is complete * in the method adopted for determining the available carbon dioxide, this determination affords a measure of gas liberated by the interaction of the acid and bicarbonate together with *half* the carbon dioxide present in the excess

* Experiments which have been made in this laboratory with prepared baking powders of known composition indicate that if the determinations are carefully carried out this assumption can reasonably be made.

of sodium bicarbonate. Hence the difference between the total and available carbon dioxide, determined as described above, gives the carbon dioxide equivalent to *half* the excess of sodium bicarbonate, or twice the difference gives the carbon dioxide equivalent to the excess of bicarbonate. The excess of sodium bicarbonate can also be obtained by direct titration (see p. 137).

Examination of the Acid.

It has already been observed that the properties of a baking powder are to some extent modified by the nature of the acid employed, and the examination and identification of the acid is second only in importance to the determination of the carbon dioxide.

Tartaric Acid.—Tartaric acid may be distinguished from cream of tarter by its solubility in alcohol, and its presence in a baking powder can be detected in the following manner.

Shake up a little of the powder with some warm alcohol, and filter off the insoluble matter (starch, bicarbonate, etc.). Test a few drops of the alcoholic filtrate with litmus solution; tartaric acid if present will give an acid reaction.

Evaporate the remainder of the alcoholic filtrate almost to dryness on a water bath, add a few drops of a concentrated solution of potassium chloride and transfer the mixture to a watch glass, add one drop of acetic acid, and stir gently with a glass rod. A fine white crystalline precipitate of potassium hydrogen tartrate (cream of tartar) will be formed —

$$HOOC(CHOH)_2COOH + KCl \rightarrow HOOC(CHOH)_2COOK + HCl.$$

Tartaric acid. Potassium acid tartrate.

Cream of Tartar.—To detect the presence of cream of tartar it is necessary to extract with water instead of alcohol. On the addition of water the cream of tartar will react with the sodium bicarbonate present, and become converted into sodium potassium tartrate thus :—

$$KHC_4H_4O_6 + NaHCO_3 \rightarrow KNaC_4H_4O_6 + H_2O + CO_2.$$

At the same time any excess of bicarbonate will go into solution. If the solution is then concentrated to a very small bulk and rendered just acid with a few drops of acetic acid,

the relatively insoluble potassium acid tartrate will separate out—

$$KNaC_4H_4O_6 + CH_3COOH \rightarrow KHC_4H_4O_6 + CH_3COONa.$$

It is important to note that this reaction depends on the presence of the *potassium* salt in the original powder. If tartaric acid only is present, this will be converted into sodium tartrate on mixing the powder with water (see equation, p. 128), and on concentrating the solution and acidifying with acetic acid no precipitate will be obtained. The tartrate can, however, be precipitated from such a solution by the addition of a potassium salt. (Compare test for tartaric acid described above.)

The test for cream of tartar may be carried out in the following manner :—

Mix a little of the powder with cold or lukewarm water, shake well to ensure the solution of the cream of tartar, and after allowing the starch to settle, decant the solution through a filter. If hot water is used the starch will gelatinise, and the mixture cannot be satisfactorily filtered.

Evaporate the filtrate almost to dryness on a water bath, acidify with a few drops of acetic acid, transfer to a watch glass, and stir gently with a glass rod.

Leach * gives the following test for the detection of tartrate present either as tartaric acid or as cream of tartar. Shake 3 to 5 gms. of the sample with 250 c.c. of water in a large flask, and allow the insoluble portion to subside. Decant through a filter, evaporate the filtrate to dryness, and to the dried residue add a small piece of resorcin and a few drops of concentrated sulphuric acid. Heat slowly in a dry test tube. A rose-red colour indicates tartaric acid or tartrate. The colour is discharged on dilution with water.

Acid Calcium Phosphate.—The presence of phosphate and calcium may be detected by applying the usual qualitative reactions. Shake up about 1 gm. of the powder with cold dilute hydrochloric acid, allow the starch to settle, decant the solution through a filter paper, and test portions of the filtrate for phosphate and for calcium as follows :—

Phosphate.—Neutralise a portion of the solution with ammonia, acidify with a few drops of nitric acid, add a considerable excess of ammonium molybdate solution, and warm. A yellow precipitate indicates phosphate.

* See list of reference books, p. 268.

Calcium.—In order to avoid the precipitation of the phosphate simultaneously with the calcium, the calcium may be precipitated as oxalate from an acetic acid solution. Nearly neutralise a portion of the hydrochloric acid solution with ammonia, then add excess of ammonium acetate and a few drops of acetic acid. From this solution the calcium can be precipitated by the addition of ammonium oxalate.

Acid Sulphate and Calcium Sulphate.—A portion of the acid solution, prepared as described above, should also be tested for sulphate by the addition of barium chloride.

If both phosphate and sulphate are present, the sulphate is probably present as an impurity in the form of calcium sulphate (see p. 131), but if sulphate alone is present, the use of an acid sulphate as part of the raising agent is indicated.

Alum.—Aluminium salts are usually tested for in the ash of the powder. If excess of alkali is present, the alumina formed during the reaction will be converted to sodium aluminate on ignition.

On extracting the ash with boiling water, filtering, and adding ammonium chloride to the filtrate the alumina will be precipitated as a white flocculent precipitate—

$$Na_2O \cdot Al_2O_3 + 2NH_4Cl + 4H_2O \rightarrow Al_2(OH)_6 + 2NH_4OH + 2NaCl.$$

Examination of the Starch.

Some of the residue left after filtering the aqueous extract of a baking powder should be examined under the microscope and the starch identified. The starch present is usually rice, maize, or potato, or mixtures of two of these.

Determination of the Acid.

If the qualitative examination indicates the presence of one acid substance only, the quantity of acid present may be calculated from the *total* carbon dioxide (which gives the total amount of sodium bicarbonate) and the *available* carbon dioxide as follows: It has already been shown that a measure of the excess of bicarbonate may be obtained with a fair degree of accuracy from the difference between the total and available carbon dioxide (see p. 133). If more accurate results are required the excess of bicarbonate may be determined by direct experiment (see below).

The difference between the total bicarbonate and the excess of bicarbonate gives the amount of bicarbonate which reacts with, or is equivalent to, the acid present; and hence from the appropriate equation the amount of acid present may be found.

This method of calculating the acid cannot be satisfactorily employed in cases where acid calcium phosphate is the acid ingredient, since not only is there a considerable variation in the composition of the acid phosphate employed, but the reacting proportions are not in most cases represented exactly by either of the equations given on page 129, and the residue may be acid in reaction though not markedly acid to taste.

The amount of tartaric acid or cream of tartar in a baking powder may also be determined by direct experiment, and this determination can be conveniently combined with the direct determination of the excess of bicarbonate.

Determination of Tartaric Acid and Excess of Sodium Bicarbonate.

The sodium and potassium salts of organic acids are converted into the corresponding carbonates on ignition. The sodium tartrate formed, on mixing a tartaric acid baking powder with water, may be converted in this manner to sodium carbonate, and the amount of carbonate thus produced determined by dissolving in water and titrating with a standard solution of an acid. (Compare examination of vinegar for mineral acids, p. 176.)

It is, of course, essential that any free bicarbonate present after treatment of the baking powder with *water*, i.e. the excess of bicarbonate, should be removed before the mixture is ignited.

This may be done by neutralising excess of bicarbonate with a mineral acid, and if a standard solution of acid is used and the volume required for neutralisation measured, the amount of free or excess bicarbonate is obtained.

Weigh out as nearly as possible 2·5 gms. of the powder into a 250 c.c. flask, add about 100 c.c. of water, shake gently, and when effervescence has ceased, dilute to 250 c.c., mix by shaking, and allow to stand until the starch has subsided. Decant the solution through a dry filter paper. To 50 c.c. of the filtrate add a few drops of litmus solution, and boil to expel the carbon dioxide. Titrate the boiling solution with twentieth normal hydrochloric or sulphuric acid.

The volume of acid used will be a measure of the excess of bicarbonate (1 c.c. N/20 acid = 0·0042 gm., $NaHCO_3$). Although the excess of bicarbonate is converted into carbonate when the solution is heated, the amount of acid required for neutralisation will not be affected by this change (see equation, p. 127).

Evaporate the neutral solution obtained in the titration to dryness in a platinum or porcelain dish on a water bath, and when quite dry carefully ignite over a flame until a well-charred residue is obtained. Extract this residue several times with hot water, filter, and wash the residue with hot water until the filtrate is no longer alkaline.

When cool, titrate the whole of the filtrate and washings with decinormal acid, using methyl orange as indicator.

The relation between the tartaric acid and the carbonate produced from it is obtained in the following manner :—

$$\underset{\substack{150\\75}}{HOOC(CHOH)_2COOH} \rightarrow \underset{\substack{194\\97}}{NaOOC(CHOH)_2COONa} \rightarrow \underset{\substack{106\\53}}{Na_2CO_3}.$$

Thus 150 parts of tartaric acid give 194 parts of sodium tartrate, which on ignition yield 106 parts of sodium carbonate.

So that for each cubic-centimetre of decinormal acid used for the titration (1 c.c. N/10 acid = 0·0053 gm. Na_2CO_3), 0·0075 gm. of tartaric acid were originally present.

The method of calculating the excess of bicarbonate and the amount of tartaric acid is illustrated in the following example.

Example.—2·5 gms. of baking powder were treated with water and diluted to 250 c.c. The solution was decanted through a filter paper. 50 c.c. of the filtrate were boiled to expel carbon dioxide, and were then titrated with N/20 hydrochloric or sulphuric acid, using litmus as indicator.

Volume of N/20 acid required = 5·6 c.c.

The neutralised solution was then evaporated to dryness, and the residue ignited, extracted with hot water and filtered. The filtrate and washings were titrated with N/10 hydrochloric or sulphuric acid, using methyl orange as indicator.

The volume of N/10 acid required to neutralise the solution = 18·0 c.c.

Excess of Bicarbonate.—1 c.c. of N/20 acid = 0·0042 gm. $NaHCO_3$.

Therefore 50 c.c. of the solution contain—

$5{\cdot}6 \times 0{\cdot}0042$ gm. $NaHCO_3$

250 ,, ,, ,, ,, $5{\cdot}6 \times 0{\cdot}0042 \times 5$,,

2·5 gms. of powder ,, $5{\cdot}6 \times 0{\cdot}0042 \times 5$,,

100 ,, ,, ,, $\dfrac{5{\cdot}6 \times 0{\cdot}0042 \times 5 \times 100}{2{\cdot}5}$

= 4·7gms. of sodium bicarbonate.

Tartaric Acid.—1 c.c. N/10 acid = 0·0053 gm. Na_2CO_3
= 0·0075 ,, tartaric acid.

And since 18·0 c.c. of N/10 acid are required to neutralise the residue from 50 c.c. of solution, therefore

50 c.c. of the solution contain —

$18{\cdot}0 \times 0{\cdot}0075$ gms. of tartaric acid

250 ,, ,, ,, $18{\cdot}0 \times 0{\cdot}0075 \times 5$,, ,,

2·5 gms. of powder ,, $18{\cdot}0 \times 0{\cdot}0075 \times 5$,, ,,

100 ,, $\dfrac{18{\cdot}0 \times 0{\cdot}0075 \times 5 \times 100}{2{\cdot}5}$

— 27·0 gms. of tartaric acid.

Determination of Tartrate in Powders containing Cream of Tartar and Mixtures of Cream of Tartar and Tartaric acid.—The method just described for tartaric acid may also be employed, with certain modifications, for the determination of cream of tartar, where this substance is used instead of tartaric acid. It may also be used for the determination of total tartrate in mixtures containing tartaric acid and cream of tartar, and for the determination of tartrates in wine.

When a cream of tartar baking powder is mixed with water, sodium potassium tartrate (Rochelle salt) is obtained, and this on ignition gives a mixture of sodium and potassium carbonates in equimolecular proportions.

$KH(CHOHCOO)_2 \rightarrow \underset{\text{Rochelle Salt}}{KNa(CHOHCOO)_2} \rightarrow \frac{1}{2}K_2CO_3 + \frac{1}{2}Na_2CO_3$

$39+1+(148)=188$ $\qquad \frac{1}{2}(78+60)+\frac{1}{2}(46+60)$

148 gms. tartrate radical $(CHOHCOO)_2$
$\rightarrow$ 60 gms. of carbonate radical (CO_3)

$\therefore$ 74 ,, ,, ,, $\rightarrow$ 30 ,, ,, ,,

(94 gms. of cream of tartar).

It will be seen that in whatever form the tartrate is present, 74 parts by weight of the tartrate radicle will on neutralisation and ignition yield 30 parts by weight of carbonate radical.

Thus each cubic centimetre of decinormal acid used for neutralisation of the ignited residue will be equivalent to 0·0074 gm. of tartrate radical, or to 0·0075 gm. of tartaric acid, or to 0·0094 gm. of cream of tartar.

So that if the acid is present entirely in the form of cream of tartar, the result can be expressed as percentage of cream of tartar. Whilst in the case of a mixture of cream of tartar and tartaric acid the results may be expressed either as tartrate radical or, if preferred, as tartaric acid.

It is sometimes convenient for practical purposes to be able to determine the proportion of tartaric acid and cream of tartar in a powder which contains them both.

Assuming that the total amount of tartrate present has been determined, and the amount of alkali required for the neutralisation of this tartrate is known, it is possible to find by calculation the proportions of cream of tartar and tartaric acid present. It should be noted that the amount of sodium bicarbonate required for the neutralisation of the acid in the powder is given by the difference between the total sodium bicarbonate and the excess of sodium bicarbonate (see p. 137).

If the acid were present entirely in the form of tartaric acid, then 74 parts of tartrate radical would require 84 parts of sodium bicarbonate for neutralisation (see equation, p. 128). On the other hand, if the acid were present entirely in the form of cream of tartar, 74 parts of tartrate radical would require only 42 parts of sodium bicarbonate for neutralisation, since the acid in this case has already been half neutralised by the potassium present in the acid salt (see equation, p. 128).

In the case of mixtures of these two acid bodies, the sodium bicarbonate required for the neutralisation of the acid will lie somewhere between these two extremes. Thus, if the amount of sodium bicarbonate required to neutralise the total acid present is known, it is possible by a simple arithmetical calculation to find the proportions of these two acid bodies present.

The following example will serve to illustrate the method of calculation.

Example.—The total carbon dioxide yielded by a baking powder was 12·1 per cent. The excess of sodium bicarbonate was 2·1 per cent., and the total tartrate radical was 22·2 per cent. It was shown by qualitative tests that both tartaric acid and cream of tartar were present. The calculation of the proportion of cream of tartar and tartaric acid present is made as follows :—

If the total carbon dioxide = 12·1 per cent., the total amount of sodium bicarbonate present = $12{\cdot}1 \times \frac{84}{44} = 23{\cdot}1$ per cent. (see p. 133).

The excess of sodium bicarbonate = 2·1 per cent.

Hence the amount of sodium bicarbonate which is required to neutralise, or is equivalent to the acid in the powder = 23·1 − 2·1 = 21·0 per cent.

If x = the number of grams of tartrate radical present as *tartaric acid* in 100 gms. of the powder, then $22{\cdot}2 - x$ = the number of grams of tartrate radical present as *cream of tartar* in 100 gms. of the powder.

It follows that since—

74 gms. of tartrate radical in the form of *tartaric acid* are neutralised by 84 gms. of sodium bicarbonate;

x gms. of tartrate radical in the form of *tartaric acid* are neutralised by $\frac{84}{74} \times x$ gms. of sodium bicarbonate.

Also since—

74 gms. of tartrate radical in the form of *cream of tartar* are neutralised by 42 gms. of sodium bicarbonate;

$22{\cdot}2 - x$ gms. of tartrate radical in the form of *cream of tartar* are neutralised by $\frac{42}{74} \times (22{\cdot}2 - x)$ gms. of sodium bicarbonate.

Then the sum of the amounts of sodium bicarbonate required for the neutralisation of the tartaric acid and cream of tartar respectively must be equal to sodium bicarbonate which is equivalent to the total acid present.

Hence—

$$\frac{84}{74} \times x + \frac{42}{74}(22{\cdot}2 - x) = 21{\cdot}0$$

$$84 \times x + 42(22{\cdot}2 - x) = 21{\cdot}0 \times 74$$

$$4x + 2(22{\cdot}2 - x) = 74$$

$$2x = 74 - 44{\cdot}4 = 29{\cdot}6$$

$$x = 14{\cdot}8$$

Thus the tartrate radical present as tartaric acid = 14·8 per cent., and the tartrate radical present as cream of tartar to 22·2 − 14·8 = 7·4 per cent.

Or since 74 parts of tartrate radical are equivalent to 75 of

tartaric acid and to 94 of cream of tartar, the amounts of tartaric acid and cream of tartar can be obtained as follows:

$$\text{Tartaric acid} = 14{\cdot}8 \times \frac{75}{74} = 15{\cdot}0 \text{ per cent.}$$

$$\text{Cream of tartar} = 7{\cdot}4 \times \frac{94}{74} = 9{\cdot}4 \quad ,,$$

Self-Raising Flours.

These are flours to which a chemical raising agent has already been added, so that the addition of baking powder or other raising agent is unnecessary. In some varieties the active agents are mixed with the wheat flour, or sometimes with other kinds of flour, e.g. cornflour, in such proportions that the self-raising flour requires to be further diluted with untreated flour before use, but more often the proportions added are such that the flour, as sold, is ready for use.

A self-raising flour may be prepared by the addition of 10 parts of sodium bicarbonate and 7·5 parts of tartaric acid (or 22·5 parts of cream of tartar) to 1000 parts of flour. These proportions allow in each case for a slight excess of the bicarbonate for "covering" (see p. 130).

Commercial preparations often contain a rather higher proportion of the active ingredients.

Acid calcium phosphate is frequently employed as the acid ingredient in self-raising flours, as owing to the large proportion of inert material (i.e. flour) present the hygroscopic properties of this salt are less likely to impair the keeping properties of the mixture than is the case with a baking powder, where the active ingredients are diluted with a relatively small proportion of starch. If a low-grade flour is used, it is possible to save the cost of the active ingredients on the price paid for the flour and to make a profit even when the self-raising flour is sold at a price only slightly above that paid for a good untreated flour.

For the purposes of making a chemical examination, a self-raising flour may be regarded as a very much diluted baking powder, so that although the methods used are the same in both cases, it is necessary in the case of self-raising flour to work with considerably larger quantities. Thus in testing a sample of self-raising flour for acid calcium phosphate, shake up about 10 gms. of the flour with cold dilute

hydrochloric acid, and then proceed in the manner described on page 135.

A portion of this solution should also be tested for sulphate (see p. 131 and also 136).

EGG POWDERS AND EGG SUBSTITUTES.

Eggs, although primarily to be regarded as a valuable form of food, may also be employed as a raising agent. Their action in this respect is dependent on their physical rather than on their chemical properties. Owing to the peculiar consistency of the egg, it is possible by "whipping" or "beating" to entangle a considerable amount of air in the egg, and it is to the expansion of this air on heating that the raising action of the egg is to be attributed.

Dried eggs may be prepared by the removal of the water in the egg by evaporation under suitable conditions. It is, of course, essential that the temperature should be kept below that at which the protein in the egg will coagulate, but even when this is the case the peculiar consistency of the fresh egg is seldom reproduced when such preparations are mixed with water. So that although dried eggs may be found of value in the kitchen for the preparation of certain dishes, they cannot as a rule be relied upon in cases where the egg has to function as a raising agent. Most of the so-called *egg powders* and *egg substitutes* which are now sold are not prepared from dried egg, and do not even remotely resemble dried egg in composition, but usually are simply baking powders to which a little yellow colouring matter has been added.

To distinguish between genuine dried egg and an "egg powder" it is only necessary to mix a little of the sample with water and heat. Genuine dried egg will "curdle" or coagulate on heating, whereas an "egg powder" will liberate carbon dioxide and the starch will gelatinise. It may be noted that, as a rule, preparations of genuine dried egg do not mix with or take up water very readily, and that in using them for cooking the best results are obtained by allowing them to steep for some hours, e.g. overnight, in cold water before use.

In making a chemical analysis of dried egg the protein, fat, and ash may be determined by usual methods employed in food analysis; whereas an "egg powder" should be treated as a baking powder. In addition, however, if desired, some examination of the colouring matter present in an egg powder

may be made. For the purposes of comparison the composition of fresh egg, dried egg, are given below :—

	Fresh Egg.	Dried Egg.
Water . .	72·8	4·8
Protein . .	11·8	43·7
Fat . . .	13·6	50·6
Ash . . .	0·95	3·7

It should be noted that in the case of genuine dried egg the protein, fat, and ash will be in approximately the same relative proportions as in fresh egg. For comparative composition of egg and custard powders see "Analyst" (1921, **46**, 271; 1922, **47**, 512; 1923, **48**, 542).

An examination of colouring matter present in several different samples of commercial egg powder should be made, since this will afford a useful practical illustration of some of the more important methods which may be employed for the detection of artificial colouring matters in food preparations in general, and a short account of these methods may conveniently be introduced here.

Artificial Colouring Matters in Foods.

The artificial colouring matters in foods may be grouped into three main classes, according to their origin :—

(I.) Naturally occurring organic colours of vegetable or animal origin.

(II.) Synthetically prepared organic colours or dyes.

(III.) Mineral or inorganic colours, pigments.

The majority of colouring matters belonging to the first class are harmless, and although opinions differ widely as to how far synthetic dyes are injurious to health, most of the dyes actually used for colouring foods are probably not harmful when added only in small amounts.

The third class includes various chromates and compounds of arsenic, copper, and other metals. Such colours cannot be considered harmless, and in many cases are distinctly poisonous (see Chap. VIII.)

The question as to how far the addition of colouring matter to foods is to be considered objectionable is not, however, entirely dependent on the nature of the colouring matter added. Harmless colours may be used to conceal inferiority or to make the preparation appear of greater value than it

really is, and the colour may then reasonably be regarded as an adulterant. In such cases it is really only necessary to prove that some artificial colouring matter is present, but it is more satisfactory to be able to show in addition to which of the three classes the added colour belongs. This can in most cases readily be done, although it may often be difficult to identify the exact colour or mixture of colours used.

Vegetable Colours.

The vegetable colours include turmeric, saffron, and annatto, and these being yellow colours might be used for colouring "egg powders." *Saffron* has a marked flavour, and except for the preparation of saffron buns and cakes is seldom used. The colour given by *annatto* is too brown in shade to be utilised in colouring egg powders, and although this substance used to be employed for colouring milk and butter, it has now been largely superseded by oil soluble coal-tar dyes.

Turmeric is not uncommonly found in egg powders, and can be identified readily by its reaction with boric acid (see p. 151).

Synthetic Colours—Coal-Tar Dyes.

So many coal-tar dyes can be used in foods that it would be quite impossible to deal with them all, especially as new colours are being frequently added to the list. Further, owing to the complex nature of most of these dyes, it is extremely difficult to devise any general system of classification, but a number of different classifications suitable for various purposes have been attempted.

Thus dyes may be classified according to their origin, e.g. aniline dyes, anthracene dyes, etc., or according to the chemical composition, e.g. nitro, azo, etc., and also according to their behaviour in dyeing, e.g. basic dyes, acid dyes.

Coal-tar dyes added to foods can as a rule be identified as such, and the class to which they belong sometimes determined, but it is often extremely difficult and in many cases impossible to identify the individual dye or combination of dyes employed. In the United States only certain specified or "permitted" dyes may be used for colouring foods. The choice of colours is thus limited and the work of the analyst much simplified.

Most of the dyes used in colouring foods are water-soluble

dyes, the majority being "acid" dyes containing one or more sulphonic acid groups ($-SO_3H$), although "basic" dyes are sometimes met with. With very few exceptions the dyes used are taken up by and dye wool directly without the addition of a mordant.

This property is made use of in examining the colouring matter in a food. A piece of white woollen cloth is boiled for five to ten minutes in an acidified solution of the colouring matter. The wool is then removed and boiled for a few minutes, first in a very dilute solution of hydrochloric acid, and then in water. If the colour is retained on the wool a dye is most probably present. Certain vegetable colours, e.g. turmeric, will also dye wool direct, but the subsequent boiling in dilute acid tends to strip the vegetable colours, and such colours can usually be distinguished from coal-tar dyes by special tests. If further information as to the nature of the dye is required, the colour is stripped from the wool by boiling with a very dilute solution of ammonia. The wool is then removed from the solution and the solution acidified with hydrochloric acid. A fresh piece of wool is introduced into the acidified solution, and the colour transferred to this by boiling.

With vegetable colouring matters this second dyeing gives practically no colour to the wool; natural fruit colours and most colours of vegetable origin remaining in the solution. The second dyeing also serves to bring out the characteristic colour of the dye, which sometimes gives rather a nondescript shade on the wool in the first dyeing, especially if vegetable colouring matters are also present. The dyed material is next subjected to a series of identification tests, and the colour classified by reference to special tables.

Full details of the methods employed are given by Green, in "Analysis of Dyestuffs" (Griffin & Co.), and also in standard works on food analysis.

Considerable experience of the methods described and of the characteristic reactions of the different classes of dyes is, however, required before reliable results can be obtained, and for practical purposes it will suffice to be able to show whether or not a coal-tar dye is present, and further identification need not be attempted.

In order to illustrate the formation of both basic and acid dyes from aniline, the composition of some of the yellow dyes such as may be used in colouring egg powders is shown below:—

$$C_6H_5NH_2 + C_6H_5NH_2 \rightarrow C_6H_5N : NC_6H_5$$

Aniline (2 molecules) — Azobenzene

$$C_6H_5N : NC_6H_4 . NH_2$$

Aminoazobenzene (Aniline Yellow)—Basic dye

Acid dye. $\leftarrow\rightarrow$ Basic dye.

$HSO_3C_6H_4 . N : N . C_6H_3 . NH_2 . SO_3H$ — $C_6H_5N : NC_6H_4N(CH_3)_2$

Aminoazobenzene disulphonic acid (Acid Yellow) — Dimethylaminoazobenzene (Butter Yellow)

Acid Dye.

$HSO_3C_6H_4 . N : NC_6H_4N(CH_3)_2$

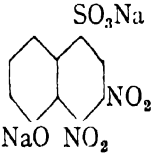
Dimethylaminoazobenzene monosulphonic acid (Methyl Orange. Heliathine)

The sulphonic acid colours are used principally in the form of their sodium salts. Thus the dye known as " Acid Yellow G " is a mixture of the sodium salts of aminoazobenzene disulphonic and aminoazobenzene monosulphonic acids. " Methyl Orange " is the sodium salt of dimethylaminoazobenzene monosulphonic acid.

These salts dissolve in water to form yellow solutions, which on the addition of acid turn red.

The basic dyes, aminoazobenzene and dimethylaminoazobenzene, are insoluble in water, but dissolve in alcohol to give a yellow solution, the colour of which changes to red on the addition of hydrochloric acid, owing to the formation of the corresponding hydrochlorides.

Mention should also be made of " Naphthol Yellow S," or the sodium salt of dinitro α-napthol sulphonic acid—



as this is one of the *permitted* dyes which may be used in the United States.

Naphthol Yellow S is soluble in water and does not turn red on the addition of hydrochloric acid, but becomes slightly paler in colour.

coal-tar dyes used those containing nitro or nitroso groups are more or less toxic. Naphthol Yellow S and Tartrazine are said to be the chief yellow colours now used in foods, and are sometimes sold under the name of "egg yellow."

According to Richardson, Naphthol Yellow S is not poisonous, since in spite of two nitro groups the presence of a sulphonic acid group causes neutralisation of toxic action.

Tartrazine or hydrazine yellow is obtained by the interaction of phenylhydrazine sulphonic acid and dihydroxytartaric acid, and contains a carboxyl as well as sulphonic acid groups. The aqueous solution of this dye does not change in colour on the addition of dilute acids, but becomes reddish brown on adding sodium hydroxide. It dyes wool directly from an acid bath.

Mineral Colours.

Mineral colours or pigments are now seldom used in colouring foods; but it may be noted that amongst a number of different samples of commercial egg powders which have been examined in this laboratory, one was found which contained lead chromate as the colouring matter (see p. 211).

The mineral colours are insoluble in water, and when mixed with food materials, the colour will be distributed in the form of small particles. These particles of pigment can usually be detected as dark coloured patches when the mixture is examined under the microscope. The chief elements of which the mineral colour is composed will be found in the ash, and such colours can thus be readily distinguished from coal-tar dyes and vegetable colouring matters, i.e. from organic colours, which are, of course, decomposed completely on ignition.

Lakes.—Lakes are insoluble pigments obtained by precipitating organic colouring matters with inorganic compounds. The precipitating agent employed varies with the nature of the colouring matter. Thus in the case of an acid colour the precipitating agent should be basic in character, and in the case of basic colour, acid in character.

The same principle is made use of in fixing dyes on textile materials by the action of a mordant.

The organic colouring matter can be liberated from these lakes by treating with acid or alkali, the reagent used depending on whether the original colour was acid or basic, and the inorganic constituents are tested for in the ash.

It is probable that in future the use of certain substances as colouring matters for foods in this country will be prohibited. As previously stated in the United States of America instead of certain colours being *prohibited*, only certain substances are *permitted*.

In the "Draft Rules" * the following list of colouring matters which may *not* be added to articles of food is given :—

1. Metallic colouring matters—Compounds of antimony, arsenic, cadmium, chromium, copper, mercury, lead, zinc.
2. Vegetable colouring matter—Gamboge.
3. Coal tar colours—Picric acid, Victoria yellow, Manchester yellow, aurentia, orange II., aurine.

Cochineal.

Cochineal, although not used in egg powders, is a natural colouring matter of special interest, since it is of animal origin, and its use for colouring foods dates back to a very early period. Cochineal is not only added to commercial food preparations, which are a comparatively recent innovation, but is also extensively employed in the kitchen for colouring jellies, creams, etc. Cochineal is sometimes incorrectly classified as a vegetable colour; it is composed, however, of the dried bodies of the insects *coccus cacti*. The insects are killed by heating and on macerating the bodies with water, a deep red-coloured liquid is obtained from which no insoluble matter separates on standing. Cochineal owes its colour to the presence of a complex acid—*carminic acid*. This colouring matter, together with other subsidiary bodies, is precipitated as a lake on the addition of alum, and in this form is sold as *carmine*. An alkaline decoction of cochineal containing a little alcohol as preservative is sold under the name of *liquid cochineal*.

Test for Cochineal.—The following test, known as the *Robin Test*, may be used for the detection of cochineal in food preparations.

Acidify the aqueous solution of the colouring matter with hydrochloric acid, and shake in a separating funnel with amyl alcohol. Cochineal imparts to this solvent a yellowish colour, the depth depending on the amount present. Wash the separated amyl alcohol with water until neutral, and divide

* Public Health (Preservatives, etc., in food) Regulations, 1925, H.M. Stationery Office, 2d.

into two portions. To one of these add a little water, and then drop by drop a solution of uranium acetate, shaking each time a drop is added. In the presence of cochineal the water is coloured a characteristic emerald green colour. To the other portion add ammonia. If cochineal is present a violet coloration is produced.

Examination of the Colouring Matter of an "Egg" Powder.

First prepare some small pieces of *white* (not cream) woollen material known as *nun's veiling*, ready for dyeing tests. Cut the fabric into strips about ¾ to 1 inch square, and free these from grease by boiling in N/100 sodium carbonate solution for a few minutes. Rinse thoroughly with hot water and the strips are then ready for use.

Extraction of the Colouring Matter with Water.

Shake up from 5 to 10 gms. of the powder with about 20 c.c. of lukewarm water, and allow to stand until effervescence has ceased and the insoluble matter has settled. Observe whether the liquid is coloured or not.

If a coloured extract is obtained, filter off the insoluble material and acidify the filtrate with a few drops of hydrochloric acid. If the colour of the solution changes from yellow to orange, or red, on the addition of the acid a dye is present, and this may be one of the sulphonic acid compounds mentioned above. If the colour becomes distinctly paler on the addition of acid, naphthol yellow may be present.

An attempt should next be made to transfer the colour to wool. Introduce one of the prepared strips of wool into the acidified solution, and boil for five to ten minutes. Remove the wool and boil it for a few minutes, first in water containing a little hydrochloric acid, and then in water only. Note if the colour is taken up by the wool.

In the case of the dyes which give a red colour in the acid solution the wool may at first be stained pink, but on washing out the acid the colour will probably change to orange or yellow (see p. 147).

Extraction of the Colouring Matter with Alcohol.

Shake up another portion of the powder with alcohol, and allow to stand until the insoluble matter has settled.

As before, note whether or not a coloured extract is obtained and also whether the alcohol appears to extract more colour than the water. If a coloured extract is obtained, filter off the insoluble matter and acidify the filtrate with hydrochloric acid. If one of the basic dyes mentioned above is present, the colour will be soluble in alcohol but not in water, and on the addition of acid to the alcoholic solution the colour will change to red or pink.

In cases where a distinctly coloured alcoholic extract is obtained, and little or no colour is extracted by water, or if dyeing from the aqueous extract is unsatisfactory, an attempt should be made to dye a strip of wool in the acidified alcoholic extract. If the alcoholic extract is distinctly yellow, but shows no change in colour on the addition of hydrochloric acid, a portion of the acidified solution should be tested for turmeric in the following manner.

Test for Turmeric.—Transfer a portion of the acidified alcoholic extract to a crucible lid, add a few drops of an aqueous solution of borax, and evaporate to dryness on a water bath. A bright red or pink residue, which turns to bluish green on moistening with alkali, shows the presence of turmeric (see p. 21).

It may be noted that turmeric is more soluble in alcohol than in water, and that naphthol yellow is more soluble in water than in alcohol.

Colours Insoluble in Water and Alcohol.—If the colour is not extracted either by water, dilute acid, or alcohol a pigment or a lake may be present.

A portion of the powder should be ashed, and the ash examined for possible metallic constituents, e.g. chromium, barium, lead.

An attempt should also be made to free any organic colour present by boiling portions with (1) hydrochloric acid, and (2) sodium hydroxide.

Some of the powder should also be examined under the microscope to see whether any deeply coloured particles of pigment can be detected.

It is important, however, to note that egg powders are frequently coloured by mixing a relatively small proportion of highly coloured starch with colourless starch to which the raising ingredients have been added. The distribution of the colour in such cases is uneven, and the appearance may suggest the presence of a pigment, but on examining such powders

under the microscope it will be seen, on carefully focussing, that the coloured patches are made up of starch grains. Whereas if a pigment or lake is present the coloured particles show up as small dark opaque masses.

"CAKE" AND "SPONGE" MIXTURES.—These mixtures are usually sold as containing the necessary dry ingredients, e.g. flour, raising agent, flavouring, and colouring matters, for making the particular preparation in question. The addition of water, or in some cases water and flour, and subsequent baking are all that should be required to complete the preparation. The colouring matter may be examined in the manner described above for egg powders, and the nature of the raising agent as described under self-raising flours. Preparations which are supposed to contain fruit flavourings, e.g. lemon sponge powders, are likely to contain citric or tartaric acid.

Chapter V.

MEAT AND MEAT EXTRACTS.

The edible flesh of any animal used for human food may be described as "meat." This term, however, is not ordinarily applied to the flesh of fish, and is more often confined to the flesh of the larger mammals, or what is usually known as "butcher's meat," e.g. mutton, beef, etc. Although the flesh of other animals and birds, e.g. poultry, game, etc., differs in some respects from that of butcher's meat, its composition is sufficiently similar for the same methods of chemical examination to be employed, and these other forms of flesh food may here be taken to be included in the term "meat."

Lean meat consists essentially of muscle fibres, connective tissue, and fat cells. The nitrogen compounds form by far the most important constituents of such meat. Carbohydrates are almost entirely lacking, and the glycogen, or animal starch (see p. 101), and muscle sugar together rarely amount to more than 1 per cent.

The nitrogen compounds consist of *proteins* and of so-called *extractives* or *meat bases*, and before proceeding further it will be advisable to give a short account of the properties and method of classification of the proteins.

PROPERTIES AND CLASSIFICATION OF THE PROTEINS.

This section is to be regarded as a summary, and is introduced mainly in order that the methods used for the examination of meat and other foods containing protein may be more clearly understood.

For further information on this subject, the student is referred to the standard text-books on organic and biochemistry.

The substances known as proteins are extremely complex nitrogeneous compounds which occur both in animals and

plants. On hydrolysis they decompose, with the ultimate formation of amino acids, and they may be regarded as condensation products of these acids.

Although a number of different nitrogeneous compounds are classified as proteins, and some of these exhibit considerable differences in chemical and physical behaviour, the chemical composition of these compounds is in most cases very similar, and may be represented as follows :—

Carbon = 50 to 55 per cent.
Hydrogen = 6·5 to 7·3 ,,
Nitrogen = 15 to 17·6 ,,
Oxygen = 19 to 24 ,,

Most varieties contain a proportion of sulphur (see p. 157), and others also contain phosphorus (see p. 157).

The proteins dissolve in water or in dilute salt solutions to form colloidal solutions, since the dissolved protein is unable to pass through animal or vegetable membranes, and the solutions show the presence of particles when observed with the ultra-microscope or in a beam of light, as in the Tyndall cone experiment. Solutions of proteins are optically active and lævro-rotatory. Many proteins are coagulated by heating their slightly acidified solutions to the boiling-point, and they are also coagulated by concentrated solutions of mineral acids and by absolute alcohol. Neutral salts when added in sufficient quantity will "salt out" or precipitate most proteins from solution, common salt and the sulphates of ammonium, magnesium and zinc being the salts most commonly used for this purpose. This "salting out" is an important aid in separating and identifying different varieties of proteins (see p. 156). As might be expected, the proteins being derived from amino acids are amphoteric in character, and are capable of forming unstable compounds both with acids and bases. Thus protein solutions are precipitated by most salts of heavy metals, e.g. copper sulphate, ferric chloride, and acidified mercuric chloride, the metal being precipitated in combination with the protein, which in this respect exhibits an acid character. On the other hand, certain weak acids, e.g. phosphotungstic acid, picric acid, and tannic acid, yield insoluble compounds with the proteins, which here behave as bases.

The formation of such precipitates as those mentioned above may be used in the testing and separating of proteins

from solution, but the proteins also give certain colour reactions on which a number of tests have been based. Among these the following may be noted :—

Millon's Reaction.—Millon's reagent is a solution of mercuric nitrate containing a trace of nitrous acid, and is prepared by dissolving mercury in concentrated nitric acid and diluting with water. With solutions of proteins Millon's reagent gives a white precipitate which becomes red on warming. This reaction indicates the presence of a phenolic group in the protein molecule, as the reagent also gives a red colour with phenol.

Xanthoproteic Reaction.—When solutions of proteins are heated with concentrated nitric acid a yellow colour is obtained, which changes to orange on the addition of ammonia to the solution. This colour is due to the presence of benzene rings in the protein molecule.

Biuret Reaction.—If a few drops of a dilute solution (2 per cent.) of copper sulphate are added to a solution of a protein in caustic soda, a red to violet coloration is obtained. The reaction derives its name from the fact that biuret, on similar treatment, gives the same colour, and the action is due to the presence of at least two groups —CO—NH— closely associated in the protein molecule.

Hydrolysis of the Proteins.

When proteins are boiled with excess of acid or alkali they cease to be coagulable by heat, and products known as *meta-proteins* are obtained. When the hydrolysis is effected with alkali the product formed is sometimes called an *albuminate* or *alkali-albumin*, and when acid is used, *syntonin* or *acid-albumin*.

Solutions of meta-proteins can also be prepared by dissolving protein which has been coagulated by heat in either acid or alkali. The meta-proteins are precipitated when the solution is neutralised.

On further hydrolysis the proteins yield a series of products which become less and less complex as the action progresses. The stages of hydrolysis may be represented thus :

Proteins → Meta-proteins → Proteoses → Peptones → Polypeptides → Amino acids.

The meta-proteins and proteoses still possess the properties of proteins, giving colloidal solutions which are precipitated by saturation with ammonium sulphate.

The peptones cannot be precipitated from solution by salts even on saturation, but they give the buiret reaction. The colour obtained with the peptones is pink, and less violet in shade than that observed in the case of the proteins.

The polypeptides may be prepared synthetically, and exhibit all degrees of complexity, the more complex giving protein reactions and the simplest forming true solutions and giving no protein reactions.

The amino acids form true solutions.

Classification of the Proteins.

The proteins, using the term in its widest sense, may be divided into the following groups :—

I. Simple Proteins.
II. Compound or Conjugated Proteins.
III. Protein Derivatives.

I. Simple Proteins.

These form by far the largest and most important group of proteins, and they may be further subdivided into a number of classes as follows :—

(*a*) *Protamines and Histones* are the simplest members of the protein group, and are basic substances which, in conjunction with nucleic acid, are found in certain varieties of fish sperm. The histones are rather more complex in composition than the protamines, but probably each class merges into the other.

(*b*) *Albumins* are soluble in water and in dilute salt solutions. Egg-albumin, serum albumin, and lactalbumin are typical examples of this class.

(*c*) *Globulins*, of which fibrinogen and serum-globulin may be taken as examples, are usually insoluble in water, but soluble in dilute salt solutions. Both the albumins and globulins are coagulated by heat, and they differ chiefly in their solubility in concentrated solutions of neutral salts.

Thus globulins may be precipitated from an aqueous solution by saturating the solution with sodium chloride or magnesium sulphate, or by half saturating the solution with

ammonium sulphate. The albumins are not precipitated under these conditions, but if the solution is saturated with ammonium sulphate, both albumins and globulins are precipitated.

(*d*) *Glutelins* are alkali soluble proteins of vegetable origin, and are closely related to the globulins. Glutenin, one of the proteins found in wheat, belongs to this class.

(*e*) *Gliadins* (*or Prolamines*) are proteins found in certain plants. They are soluble in alcohol. The most important member of this class is the *gliadin* of wheat. The *glutenin* and *gliadin* of wheat together form the substance known as *gluten*, on which the characteristic properties of wheat flour depend (see p. 122).

(*f*) *Scelero-proteins*, which were formerly called *albuminoids*, form a group of proteins obtained from various sources, such as horn, hair, silk, gelatine, etc. They are derived, as the name indicates, chiefly from connective and supporting tissues, and have no common distinguishing properties.

Collagen, the substance of which the white fibres of connective tissue in meat are composed, yields, on boiling with water, *gelatine*. This latter substance has the property of setting to form jelly when a solution prepared with hot water is allowed to cool, and physically it is regarded as a typical colloid of the *reversible* type.

Elastin, the substance of which the yellow or elastic fibres are composed, is characterised by its insolubility in water and in salt solutions.

Keratin, which is found in the surface layers of the epidermis in hair, nails, hoofs, and horns, is also insoluble, and differs from the other proteins in containing a high percentage of sulphur (compare Wool, Vol. I., Chap. IV.).

II. Compound or Conjugated Proteins.

This class contains substances in which the protein molecule is united with other complex groups, so that on hydrolysis other products in addition to amino acids are obtained. Thus the *nucleoproteins* are compounds of proteins with nucleic acid, whilst the *hæmoglobin* of the blood is a compound of a protein with an iron-containing substance *hæmatin*.

The *phosphoproteins* of which the most important are the *caseinogen* of milk and *vitellin* from egg-yolk, yield phosphoric

acid on gentle hydrolysis with alkali; but it is not quite certain to which group of proteins these latter substances really belong, and they are sometimes included as one of the classes of the Simple Proteins.

III. Protein Derivatives.

These are various products, meta-proteins, proteoses, peptones, and polypeptides, obtained by the hydrolysis of the proteins (see p. 155).

Meat Bases or Extractives.

Meat, in addition to containing nitrogen in the form of protein and gelatine, also contains other nitrogeneous bodies, which are usually classified as *meat bases* or *extractives*. These include such substances as creatine, creatinine, carnosine, methyl guanidine, and certain purine bases. It is these extractives which give the characteristic taste and flavour to the meat, and which are chiefly responsible for the stimulating properties of "meat extracts." They are, however, lacking in food value, and do not rank as foods.

The Chemical Examination of Meat.

The chief characteristics of the flesh of various animals are in the main very similar, whatever the species of the animal, and it is usually impossible from a chemical analysis to distinguish particular kinds of flesh when mixed with that of other animals in such preparations as sausages and potted meats.

Characteristics of Fresh Sound Meat.

The reaction of the meat should be acid. If it is neutral or alkaline, decomposition is indicated, except in cases where the alkalinity is due to the addition of alkaline salts, e.g. borax, as preservatives. The meat should be neither pale pink nor purple in colour, and should have a marbled appearance due to the presence of small veins of fat distributed among the muscles.

In consistency it should be firm and elastic to the touch, and should hardly moisten the finger. The meat should be practically free from odour, and on standing for a day or two

it should not become moist but, on the contrary, should grow drier.

When dried at 100° C. it should not lose more than 70 to 74 per cent. in weight, and should shrink very little on cooling. Unsound meat frequently loses 80 per cent. or more on drying.

Methods of Examination.

Chemical methods are only rarely applicable to the examination of fresh meat. Meat inspection is a matter for the food inspector rather than for the analyst, and the methods here described are more usually employed for the examination of various forms of preserved meat, e.g. meat preserved in tins or in glass, potted meat, sausages, etc.

In cases where for special reasons—such, for example, as the compilation of data on the composition of different meats—it is necessary to undertake the examination of fresh meat, due regard must be paid to the perishable nature of the material. The determination should be begun immediately on the receipt of the sample and carried out as rapidly as possible. If delays cannot be avoided, the sample as well as portions of the solution prepared from it in the earlier stages of the examination, should be kept on ice. Even at low temperatures changes due to both bacterial and enzyme action take place, and the nature of the proteins is slowly changed.

Preparation of the Sample.

The edible portion of meat should first be separated from the bone and gristle with a sharp knife. The edible meat may then be divided into *visible fat* and *lean meat*, and each of these portions weighed.

The lean meat is finely chopped or passed through a mincing machine until it is reduced to a finely divided homogeneous mass. Portions of this meat may then de used for the following determinations :—

Moisture and Fat.

Moisture.—Weigh out 2 to 5 gms. of the meat into a weighed porcelain dish, and dry in a steam oven until a constant weight is obtained.

A slight oxidation of the fat on heating may introduce a

small error, but except in cases where great accuracy is required, this possible error may be neglected.

Fat.—The dried residue is next extracted with ether to remove the fat; this operation is usually carried out in a Soxhlet extractor (see p. 8).

Transfer the dried meat to a special extraction thimble (made of filter paper), or if one of these is not available, fold up the meat carefully in a piece of fat-free filter paper (i.e. filter paper which has been previously extracted with ether), and introduce it into the extraction tube.

Connect up the extraction tube with a weighed flask containing ether, and with a condenser (see Fig. 4). Heat on an electric hot-plate (or water bath) for several hours.

Distil off the ether in the flask, and dry the residue in a steam oven to a constant weight.

The complete extraction of the fat is often a matter of some difficulty, and may be facilitated by grinding the dried meat with dry sand in a mortar and then extracting this mixture with ether.

Ash.

Dry another portion of the meat (2 to 5 gms.) in a platinum dish in a steam oven. Ignite over a Bunsen flame until an ash of uniform colour is obtained, cool in a desiccator, and weigh.

Separation and Examination of Nitrogenous Compounds.

The question as to how far the nitrogenous bodies in the meat are to be subdivided for analysis will depend largely on the purpose for which the analysis is required, but as a rule it is only necessary to divide these bodies into several main groups according to their solubility in water and behaviour on heating. The amount of nitrogen present in each group can be determined separately by a Kjeldahl estimation, and, if required, the corresponding nitrogen substance can in each case be obtained by multiplying by the appropriate factor.

I. Determination of Total Nitrogen.

Weigh out 1 gm. of meat into a Kjeldahl flask and proceed in the manner described for the determination of total protein

in milk (see p. 11). Calculate the percentage of nitrogen in the meat. For protein factor to be used see p. 163.

II. Determination of Nitrogenous Substances Soluble in Cold Water.

A portion of the meat is extracted with cold water, which removes the soluble proteins (soluble globulins, albumins, proteoses, and peptones) and also the meat bases; leaving behind the insoluble globulins and sclero-proteins (collagen, elastin, etc.). The nitrogen present in an aliquot portion of the extract is determined, and from this the percentage of nitrogen which is present in the form of substances which are soluble in cold water is obtained.

The determination of the nitrogen in the cold water extract is carried out as follows: Weigh out 10 gms. of the minced meat into a mortar, mix with about 2 gms. of sand, and grind well. Add 50 c.c. of cold water, mix thoroughly, and then allow to stand for one hour.

Filter the solution through a little cotton wool contained in the apex of a glass filter funnel, and collect the filtrate in 100 c.c. graduated flask.

Owing to the colloidal character of the solution, filtering through a filter paper is extremely slow, and if a little care be exercised it will be found that the sand and cotton wool will effectively retain all the solid particles.

Press well the residue left in the mortar, and pour off as much of the liquid as possible through the filter.

Mix the residue with 30 c.c. of fresh water, and again allow to stand for one hour. Filter off the liquid through the same filter as before, and take care to press the residue well in order to separate the liquid as completely as possible. Wash the residue on the filter with a little cold water (about 10 c.c.), make up the combined filtrates to 100 c.c., and mix the solution by shaking. Pipette out 25 c.c. of the solution into a Kjeldahl flask and determine the amount of nitrogen present. Calculate the nitrogen which would be present in 1000 c.c. of the solution, i.e. the soluble nitrogen which would be obtained from 100 gms. of meat.

The nitrogen present in the substances which are insoluble in cold water is given by the difference between the total nitrogen and the nitrogen soluble in cold water (I.—II.).

III. Determination of Coagulable Protein Nitrogen.

The coagulable albumins and globulins can be precipitated from the cold water extract by boiling, and may thus be separated from the proteoses, peptones, and meat bases which remain in the solution.

Take 50 c.c. of the cold water extract prepared as described above, add a few drops of acetic acid, and boil until the coagulable proteins separate. Filter through a filter paper, and wash the insoluble material several times with hot water.

Transfer the filter paper with its contents to a Kjeldahl flask and determine the amount of nitrogen present. Calculate, as before, the nitrogen which would have been obtained from 1000 c.c. of the solution, i.e. from 100 gms. of meat.

The difference between the soluble nitrogen and the coagulable protein nitrogen (II.—III.) will give the nitrogen present in the form of proteoses, peptones, and meat bases.

Nitrogen Present in Other Forms.

In carrying out a chemical examination of the nitrogenous substances present in meat, it is rarely necessary to do more than determine the amounts of nitrogen present in the three main groups as described above, but further subdivisions may be made if required.

Thus from the filtrate obtained after the removal of the coagulable protein (III.) the proteoses may be precipitated by the addition of zinc sulphate, and separated from the peptones and meat bases which remain in solution.

No really satisfactory method of separating the total meat bases from the peptones has yet been devised, but the amount of creatin and creatinine can be determined by a colorimetric method (see p. 170), and it is also possible to determine the total purine bases present.

The insoluble material, consisting largely of connective tissue, which is left after extracting with cold water (II.), can be boiled with water to convert the collagen to gelatine, so that the nitrogen present in this form can be found by making a nitrogen determination on this hot water extract. For further details of methods used in making these separations, see Leach, " Food Inspection and Analysis."

Factor for Conversion of Nitrogen to Protein.

Although the nitrogen in meat is not present entirely in the form of protein, and the amount of nitrogen in individual proteins (compare casein in milk, p. 11) also varies slightly, the customary method of taking percentage of nitrogen × 6·25 as representing protein, gives, in the case of meat, a fairly close approximation to the amount of total nitrogenous substance present. This factor (6·25), which is based on the assumption that the protein contains 16 per cent. of nitrogen (see p. 154), may therefore be used for the conversion of nitrogen to protein in each of the three above groups.

In determinations of the amount of nitrogen present as collagen and gelatine the factor 5·55 should be used, and in the case of solutions or preparations known to contain meat bases only the factor 3·12 is usually employed.

Preservatives in Meat.

The preservatives most generally used in the case of meat are boric acid, or borates, and sulphites; the methods of examining meat for these substances is described later (see p. 165, and also p. 202).

When meat is "cured" or "pickled" by treatment with strong salt solution a little saltpetre (potassium nitrate) is usually added to preserve the natural colour of the meat, which is to a great extent destroyed by the action of the salt. In the case of meat which has been treated in this manner, it is necessary to modify slightly the method ordinarily used for the determination of total nitrogen.

Meat is now largely preserved by keeping in cold storage (see p. 204).

Examination of Sausages.

Since sausages represent a form of raw meat which can be readily adulterated, the methods used for their examination may be briefly described here. Sausages are made from finely minced meat, seasoned and stuffed into cases which are prepared from the cleaned intestinal skin of animals.

Although a great number of different kinds of sausages can be prepared by varying the nature of the meat and seasonings used, the sausages prepared in this country are usually made either of pork or of beef. Sausages are sometimes

artificially coloured, and often contain bread crumbs, flour, or other starchy material. This increases the water-absorbing power of the meat, so that the meat can be further adulterated by the addition of water, whilst the gelatinisation and consequent swelling of the starch on heating will tend to disguise any undue shrinkage of the meat on cooking.

The various determinations previously described in connection with the examination of meat can also be carried out in the case of sausages, but, in addition, the sausages should be examined for added starch, for colouring matter, and also for preservatives. A method of determining the percentage of meat in sausages is described on page 166.

Detection of Starch in Sausages.

A portion of the sausage meat should be boiled with water for a few minutes and then cooled. The extract is then tested for starch by the addition of iodine in potassium iodide, and if starch is present in considerable quantity the characteristic blue colour will be obtained. Small admixtures of starch may be detected by teasing out a little of the sausage on to a slide, adding a drop of dilute iodine solution and examining under the microscope (see p. 101). Very small amounts of starch may be introduced in the pepper added for flavouring, and the microscopical examination should show whether the starch is present in this form or whether starch from cereals is also present (see Plates II. and III., pages 100 and 237, and also p. 255.)

Artificial Colouring Matters in Sausages.

Freshly chopped meat rapidly changes in colour, and potassium nitrate is often added to prevent this loss of colour (see p. 163), whilst the sulphites which are sometimes used as preservatives also help to retain the colour. Artificial colouring matters of various kinds are also not uncommonly mixed with the meat. Oxide of iron, synthetic aniline dyes, e.g. aniline red, and sometimes cochineal are used for this purpose.

Oxide of Iron or Red Ochre.—This can be detected by an examination of the ash. Fresh meat gives an ash which contains only a trace of iron, and this will be insufficient to colour the ash, whereas if an appreciable quantity of iron oxide has been added the ash will exhibit a characteristic red-brown colour.

Aniline Dyes and Cochineal.—The general methods used for examining foods for aniline dyes have already been discussed (see p. 146), and these may be applied to the examination of sausage meat. Many of the colouring matters used can be extracted with alcohol slightly acidified with hydrochloric acid. This alcoholic extract should be filtered, concentrated, and then boiled with a piece of white wool. If the wool is distinctly coloured, a dye is present.

If the meat is treated with 50 per cent. alcohol and well shaken at intervals for about two hours, the natural colouring matter of the meat is almost completely decolorised, whereas if artificial colouring matter has been added, the meat usually still retains a considerable amount of colour after this treatment. It is important to note that if the meat has been treated with potassium nitrate a red substance is often extracted with ether, or with alcohol and ether, even when other colouring matter is absent. It has been shown that the artificial colouring matters which are usually added to sausages, e.g. aniline red and cochineal (carmine), can be extracted by warming the finely divided material with a 5 per cent. solution of sodium salicylate for a short time on a water bath. On adding ammonia to the extract, red precipitates are thrown down which contain the colouring matter.

A special test for cochineal is described on p. 149.

Preservatives in Sausages.

As already stated, the preservatives most usually added to meat products are boric acid, or borates, and sulphites. It is probable that in future sulphurous acid and sulphites only will be allowed in proportions not exceeding 3 grains of SO_2 per lb. (see p. 202).

Boric acid and borates may be tested for in the following manner. Mix about 25 gms. of sausage meat with 50 c.c. of water in a mortar. Boil the mixture in a flask for a few minutes, cool, and filter through a wet filter paper to remove meat fibres and fat. Acidify the filtrate with hydrochloric acid and test with turmeric paper (see p. 21).

Small quantities of borates can be more readily detected in the ash of the meat, but in this case the meat must be treated with lime-water, before ashing, in order to fix the boric acid. The ash is extracted with a small volume of hot water acidified with hydrochloric acid, and filtered, the filtrate

is evaporated to dryness on a crucible lid with a few drops of an alcoholic turmeric solution (see p. 21).

Sulphites.—The usual method for the detection and determinations of sulphites in meat and other foods consists in treating the material with phosphoric acid to liberate the sulphur dioxide. The mixture is then distilled in a current of carbon dioxide, the gases are passed into water containing bromine, and the sulphur dioxide is thus oxidised to sulphuric acid. The excess of bromine is removed by boiling, and the sulphate determined gravimetrically by precipitation as barium sulphate in the usual manner.

In the case of meat, and also of other food products (see p. 236 and p. 239), volatile sulphides are formed as decomposition products on heating. So that, if the process described above yields only very small quantities of sulphate, it does not necessarily follow that sulphites have been added to the meat. Chapman ("Analyst," 1922, **47**, 204) recommends the use of hydrogen peroxide instead of bromine, as with this reagent sulphur dioxide is oxidised but not volatile organic sulphur compounds.

In a series of experiments which have been carried out recently in this laboratory, dealing with the disappearance of sulphite preservatives from meat exposed to air and on cooking, it has been found that concordant results are obtained if the distillate passes into a solution of hydrogen peroxide, and the sulphuric acid produced is titrated with N/10 sodium hydroxide solution, using methyl orange as indicator.

For making a qualitative test only, a test paper which has been prepared by treating filter paper with starch solution and with a solution of sodium iodate (containing citric acid) may be used. On bringing the meat into contact with this paper the sulphite, if present, will cause the liberation of iodine from the iodate, and the starch will be turned blue.

Estimation of Meat in Sausages and Meat Pastes.

A method for the approximate determination of the quantity of meat in sausages and meat pastes has been suggested by Stubbs and More ("Analyst," 1919, **44**, 125). This method is based on the following considerations:—

(1) Meat (beef, mutton, or pork) is free from carbohydrate and crude cellulose matter, and contains a fairly uniform percentage of nitrogen calculated on fat-free meat. The average percentage in beef and mutton is 3·75, and in pork 4·0.

(2) The substances used as "fillers" contain, when in a condition suitable for mixing, about 40 per cent. of water and, with the exception of soya meal, about 50 per cent. of carbohydrate and crude cellulose matter, and about 1 per cent. of nitrogen.

The percentages of fat, protein, and ash are determined in the usual manner.

The total amount of non-fatty solids must also be determined. For this purpose a weighed and dried portion of the meat is extracted with ether until free from fat. This solid residue, dried to a constant weight, gives the non-fatty solids.

The amount of crude cellulose, carbohydrate, etc. (i.e. "filler" in the dry state), is then obtained by deducting from the percentage of non-fatty solids the sum of the percentages of protein and ash.

If this amount is multiplied by two the approximate percentage of bread or cereal "filler," in the condition in which it is used for mixing, is obtained.

One per cent. of the "filler" is taken as the nitrogen due to the "filler." If this quantity is deducted from the total nitrogen the balance of nitrogen which is due to the meat is obtained.

To obtain the *percentage of defatted meat*, the meat nitrogen is multiplied by $\frac{100}{3{\cdot}75}$ in the case of beef or mutton, or by $\frac{100}{4{\cdot}0}$ in the case of pork, or by $\frac{100}{3{\cdot}87}$ in the case of mixed meats.

The *total percentage of meat* in the sample is the sum of the percentages of fat and of defatted meat as obtained above.

The possible addition of a substance such as ammonium sulphate, in order to increase the apparent percentage of meat, should be borne in mind.

Potted Meats, Galantines, etc.

These are preparations made from cooked meat; so that the proportion of nitrogen soluble in cold water will be low, and it is unlikely that the cold water extract will contain any appreciable quantity of coagulable protein.

The examination for starch, colouring matter, percentage of meat, and preservatives should be carried out as described above.

MEAT EXTRACTS AND MEAT JUICES.

Meat extracts, as the name implies, are prepared by extracting meat with water, but such extracts vary considerably in composition according to the method of preparation used.

Extracts, which have been prepared by extraction with water at a temperature not exceeding 75° C., will, in addition to proteoses, peptone, gelatine, and meat bases, contain a proportion of coagulable proteins, e.g. albumins and globulins. If, on the other hand, boiling water is used the proportion of gelatine and also of meat bases may be increased, but the coagulable proteins will have been rendered insoluble, and will not be present in the extract, though they are sometimes mixed in with the extract, forming an insoluble sediment.

The excess of water in the extract is removed by evaporation, and if coagulation of the proteins is to be avoided the evaporation must be carried out at a low temperature, and vacuum pans are generally used for this purpose.

The water content is reduced to about 50 per cent. for liquid extracts, and to 18-25 per cent. for pasty extracts.

It is of interest to note that Liebig in preparing his original extract used cold water, but afterwards advocated the use of boiling water, and it is perhaps necessary to point out that the term "Liebig" is not a proprietary one, and may at the present time be used in connection with any process.

In the United States meat extracts are often prepared as a by-product of the canning industry, simply by evaporating the liquor in which the meat used for canning has been cooked.

Although by careful methods of preparation it is possible to obtain meat extracts containing some food material in the form of coagulable protein, it is generally agreed that such preparations are to be regarded primarily as food adjuncts or stimulants, which, though of distinct value in the diet, should not be ranked as foods.

Meat juice is the fluid portion of the muscle fibre which has been expressed from the meat by pressure or otherwise, and this may be concentrated by evaporation at temperatures below that of the coagulating point of the coagulable protein. The nitrogenous bodies in the liquid should contain not less than 35 per cent. of coagulable proteins.

Examination of Meat Extracts and Meat Juices.

As in the examination of meat the determinations to be made, and more especially the extent to which the nitrogenous bodies present are to be subdivided, will depend largely on the purposes for which the examination is undertaken. In this connection it is necessary to point out again that there is no really satisfactory method of separating the meat bases, as such, from the other nitrogenous bodies left in the solution after the removal of the coagulable proteins and proteoses.

Where some information as to the general character of the sample is required, an examination on the following lines should suffice.

Qualitative Examination.

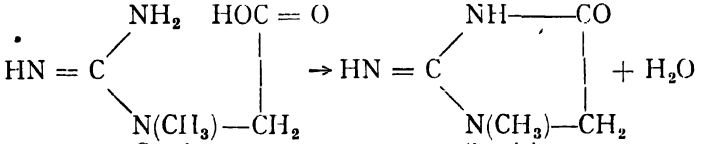
(I.) *Insoluble Material, Meat Fibres, etc.*—Mix some of the extract with cold water and note if it is completely soluble. If the solution is not clear, filter. The residue may be examined under the microscope for the presence of meat fibres.

(II.) *Coagulable Protein.*—To the filtrate obtained above add a few drops of acetic acid, boil for a few minutes, and note if any coagulation occurs, which will be the case if albumins are present. Again filter the solution.

(III.) *Proteoses and Gelatine.*—To a portion of the filtrate obtained from (II.) add two or three drops of dilute sulphuric acid, and then some finely powdered crystals of zinc sulphate. Stir well, and continue to add the salt until no more will dissolve. Allow the solution to stand for some time, and note whether any precipitate is formed, owing to the separation of proteoses and gelatine.

(IV.) *Creatine and Creatinine.*—The detection of these substances is of special importance in relation to the possible use of yeast extracts as substitutes for meat extracts. Genuine yeast extracts contain various purine bases, but creatin and creatinine are absent.

Creatine, on boiling with acids, is converted into its anhydride creatinine :—



On the addition of picric acid to a solution containing creatinine and sodium hydroxide, a deep orange-red colour is obtained (Jaffé Reaction). To test for creatinine in a meat extract, boil another portion of the filtrate obtained from (II.) with a few drops of hydrochloric acid, to convert the creatin to creatinine. Make the solution distinctly alkaline with sodium hydroxide, and then add to the solution about an equal volume of a saturated solution of picric acid in water.

This reaction may also be used for the quantitative determination of creatinine by working with an aliquot portion of the filtrate obtained from a known weight of the sample. The colour obtained is then matched in a colorimeter against that of a solution of potassium dichromate which has been previously standardised against a creatinine solution of known concentration. (For particulars of the method see "Food Inspection and Analysis," by Leach.)

Quantitative Examination.

If a weighed quantity of the sample is used, nitrogen determinations may be made on the washed residues collected in (I.) and (II.) above, and in this way a measure of the *insoluble fibre* and of the *coagulable protein* is obtained. *Alternatively*, the residues obtained in (I.) and (II.) may be collected on dried, weighed filter papers, and after the necessary washing these may be dried in a steam oven and weighed. These dried residues are then ashed, and the weight of ash deducted from the total weight of the residue. These nett weights can then be returned as *meat fibre* and *coagulable protein* respectively.

In carrying out such determinations, from 8 to 10 gms. of extracts of a pasty consistency, and from 20 to 25 gms. of liquid extracts should be used.

Preservatives and Salt.

Preservatives are not often added to meat extracts, but boric acid and sulphite may be tested for as directed under sausages (p. 165). Common salt in considerable quantities may be present, and should be looked for in the ash.

Chapter VI.

VINEGAR, FRUIT JUICES, AND VEGETABLE ACIDS.

VINEGAR.

Genuine "vinegar" is a product obtained by the oxidation of wine or other fermented sugar solutions (i.e. solutions originally containing sugar which have undergone alcoholic fermentation), and the characteristic constituent is acetic acid.

The chemical changes which take place in the conversion of sugar to acetic acid can be shown by the following equations:—

(1) $C_{12}H_{22}O_{11} + H_2O \rightarrow 2C_6H_{12}O_6$
Sucrose. Invert sugar.

(2) $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$
Alcohol.

(3) $C_2H_5OH + O \rightarrow CH_3 . CHO + H_2O$
Acetaldehyde.

(4) $CH_3 . CHO + O \rightarrow CH_3COOH$
Acetic acid.

The reaction between free oxygen and alcohol takes place under the influence of platinum black, and also of some other bodies, but the production of vinegar from alcoholic liquids is usually brought about by a fermentation process, i.e. by the action of certain micro-organisms (see p. 106). Although the methods used for the production of vinegar may vary in some respects with the nature of the liquid which is to be employed, the principles of the process are in all cases the same.

The alcoholic liquid is brought into contact with absorbent material saturated with old vinegar, and therefore rich in acetic acid forming bacteria, whilst an adequate supply of air is ensured by special mechanical arrangements. Besides acetic acid, vinegar often normally contains small amounts of other organic acids, sugars, dextrin, and colouring matters.

Sometimes caramel is added as colouring matter if the vinegar first obtained is too light in colour.

The aromatic odour is to be attributed to esters, and is sometimes imitated by the addition of ethyl acetate. The different varieties of vinegar derive their names from the nature of the liquid used for their preparation, and the principal characteristics of the more important of these may be briefly described here.

Malt Vinegar.—In this country malt vinegar is used more extensively than any of the other varieties. It is prepared from a liquid obtained by mashing malt (see p. 104), or malt and other cereals, with water. This liquid is fermented by the action of yeast (see p. 106), and then oxidised as described above. On evaporation malt vinegar yields a glutinous residue which contains appreciable quantities of phosphates and some nitrogenous matter. The total solids usually amount to from 4 to 6 per cent., and the acetic acid from 3 to 6 per cent.

Wine vinegar may be prepared from either red or white wine, and will vary slightly in composition with the nature of the wine used. In this class of vinegar the residue obtained on evaporation usually amounts to from 1·5 to 2·5 per cent. On extraction with alcohol, the whole of this solid matter dissolves with the exception of a small residue of acid potassium tartrate, the presence of which is characteristic of wine vinegar.

Normal wine vinegar may contain from 6 to 12 per cent. of acetic acid, the amount usually present being between 6 and 8 per cent.

Cider vinegar, which is much more commonly used in the United States than in this country, normally contains a proportion of malic acid. The total solids average from 1·5 to 3 per cent., and the acetic acid from 3 to 6 per cent.

A number of other kinds of vinegar can be prepared, e.g. *sugar or molasses* vinegar, *glucose* vinegar, and *distilled or spirit* vinegar (prepared by the oxidation of dilute solutions of alcohol). All these preparations are lacking in flavour and in solids, and are used chiefly as adulterants of the better known varieties.

Wood vinegar is a term which is sometimes applied to solutions of acetic acid prepared by purifying the crude pyroligneous acid which is obtained by the distillation of wood. In this case the acetic acid is not obtained by the oxidation of alcohol, and such solutions should not, in the ordinarily accepted sense of the word, be classified as *vinegar*.

EXAMINATION OF VINEGAR.

From the foregoing observations it is clear that in examining a sample of vinegar primary importance attaches to the determination of the amount of acetic acid and to the amount of solid matter present in the solution. In addition, the residue obtained on evaporation should be examined for the presence or absence of certain characteristic substances, e.g. phosphates in the case of malt vinegar, and tartrates in the case of wine vinegar. It will also be necessary to determine whether the acid is present entirely in the form of organic acid, or whether mineral acid has been added.

Determination of Total Solids.

Shake the sample well, and by means of a pipette transfer 10 c.c. to a weighed dish. Evaporate on a water bath to a syrupy consistency, and then dry in a steam oven for two to three hours and weigh.

Difficulty is sometimes experienced in removing the last traces of acetic acid by evaporation, and if this is the case, the residue may be treated with a little alcohol and again evaporated to dryness and weighed.

Calculation of Results.—In making an analysis of vinegar, it is usual to express the results as percentages by *weight;* but for most purposes cubic centimetres may be taken as equivalent to grams, i.e. 100 c.c. of the liquid may be regarded as 100 gms. If greater accuracy is required the liquid should be weighed out, or the specific gravity (usually 1·01 to 1·02) may be found by means of a Westphal balance or hydrometer, and the volume of liquid used multiplied by the specific gravity to give the exact weight (compare Milk, Chap. I.).

Examination of Residue.

Alkalinity of Ash and Phosphates.—Carefully ignite the residue from the determination of the total solids over a flame or in a muffle furnace. Add a few drops of water to the ash and boil. Test the solution with red litmus paper, and note if an alkaline reaction is obtained. If the solution is alkaline the vinegar is free from mineral acids (see p. 176) Next acidify the solution of the ash with a few drops of nitric acid and filter. To the filtrate add a considerable excess of ammonium molybdate and warm to precipitate the phosphate.

A normal malt vinegar usually contains from 0·05 to 0·1 per cent. of phosphoric acid.

Tartrates.—In the case of a wine vinegar, or where there is reason to suppose that malt or other vinegar has been mixed with wine vinegar, another portion of the vinegar should be evaporated to dryness, and the residue tested for the presence of acid potassium tartrate.

Treat the dry residue with absolute alcohol, and heat on a water bath for a short time, until no more of the solid matter will dissolve. Carefully pour off the alcoholic solution and dissolve the residue in the least possible quantity of hot water. Add a drop of acetic acid, transfer to a watch-glass, and precipitate the acid potassium tartrate by gently stirring (see p. 134).

Tartaric acid is occasionally added to vinegar as an adulterant, and in this case the residue obtained on evaporation will be viscous and acid. The free tartaric acid will dissolve on extraction with alcohol, and the presence of tartaric acid in the alcoholic solution may be shown in the manner described under " Baking Powder " (see p. 134).

Total Acidity.

The acidity of the vinegar can be determined by direct titration with standard alkali, using phenolphthalein, and, unless mineral acid is present, the result may be returned as acetic acid.

By suitably diluting the vinegar before titration, any interfering colour can be reduced.

Dilute 10 c.c. of vinegar to 100 c.c. in a graduated flask, and titrate portions of 25 c.c. with decinormal sodium hydroxide solution, using phenolphthalein as indicator.

Calculate the acidity as grams of acetic acid in 100 c.c. of vinegar. From the equation—

$$\underset{60\text{ gms.}}{CH_3COOH} + \underset{40\text{ gms.}}{NaOH} \rightarrow CH_3COONa + H_2O$$

it will be seen that 1 c.c. of N/10 sodium hydroxide is equivalent to 0·006 gm. of acetic acid.

In works or factories where determinations of acidity of a large number of vinegars have to be carried out as a routine process, the method can be simplified in various ways.

Thus, if 6 c.c. of vinegar are titrated with decinormal

sodium hydroxide solution, the number of cubic centimetres of the alkali required, divided by 10, gives the percentage of acetic acid. For, supposing 48·6 c.c. of N/10 sodium hydroxide were used to neutralise 6 c.c. of vinegar, then

6 c.c. of vinegar contain $48{\cdot}6 \times 0{\cdot}006$ gm. of acetic acid

or 100 c.c. " " $\frac{48{\cdot}6 \times 0{\cdot}006 \times 100}{6}$ " . "

$= \frac{48{\cdot}6 \times 0{\cdot}6}{6}$ " "

$= 48{\cdot}6 \times 0{\cdot}1$ " "

$= 4{\cdot}86$ " "

Also, since the preparation and standardisation of the sodium hydroxide solution in some cases presents a difficulty, a *saturated* solution of lime is sometimes substituted for the decinormal alkali solution. The lime water, if saturated, will have an *approximately* constant concentration, and its normality may be taken as N/21·4.

Then, since a normal solution of acetic acid contains 60 gms. per litre, 1 litre of lime water will be equivalent to

$$\frac{60}{21{\cdot}4} = 2{\cdot}8 \text{ gms. of acetic acid,}$$

or 1 c.c. of lime water = 0·0028 gm. of acetic acid.

If the titration is in this case carried out on 2·8 c.c. of vinegar, measured out from a burette, then, as before, the number of cubic centimetres of the alkaline solution required for neutralisation divided by 10, gives the number of grams of acetic acid in 100 c.c. of vinegar. The strength or acidity of vinegar is sometimes expressed by numbers, 18, 20, 22, and 24 being those most commonly sold. These figures refer to the number of grains of dry sodium carbonate required to neutralise 1 oz. of vinegar.

These strengths may be converted to percentages in the following manner :—

If the vinegar has a strength of " 24," then 1 oz. of vinegar is neutralised by 24 grains of sodium carbonate. 53 parts by weight of sodium carbonate are equivalent to 60 parts by weight of acetic acid ; so that—

1 oz. of vinegar contains $24 \times \frac{60}{53}$ grains of acetic acid.

Or since 1 oz. = 437·5 grains,

1 oz. of vinegar contains $24 \times \frac{60}{53 \times 437{\cdot}5}$ ozs. of acetic acid.

$\therefore$ 100 ozs. of vinegar contain $24 \times \frac{60 \times 100}{53 \times 437{\cdot}5}$ ozs. of acetic acid.

$= 24 \times 0{\cdot}259$. " "

$= 6{\cdot}2$ ozs. " "

Or the vinegar contains 6·2 per cent. of acetic acid.

Thus it will be seen that the "number" of the vinegar, multiplied by the factor 0·259, will give the percentage of acetic acid.

Mineral Acids in Vinegar.

Weak vinegar is liable to putrefy on keeping, and by an old excise regulation the addition of 1 gallon of sulphuric acid to 1000 gallons of vinegar is permitted. Such an addition is unnecessary in the case of good vinegar, and is not often practised, so that at the present time mineral acids are seldom found in vinegar.

It has been thought advisable, however, to include some description of the methods which can be used for the detection of mineral acids in vinegar, as the principles involved are in some cases also applicable to the examination of other preparations containing mixtures of organic and mineral acids.

In the case of vinegar, the ordinary qualitative tests for sulphate and chloride cannot be taken as evidence that the corresponding acids are present, since the alkali metal salts of these acids are normal constituents of vinegar.

Vinegar also contains small amounts of the sodium and potassium salts of acetic acid, and possibly of other organic acids, e.g. tartaric or malic acid. Mineral acids, even if present only in small quantities, will decompose such salts, converting them to chlorides or sulphates, as the case may be, and the free mineral acid, as such, will disappear with the liberation of an equivalent amount of organic acid.

$$CH_3COONa + HCl \rightarrow CH_3COOH + NaCl.$$

Any mineral acid in excess of that required for such reactions will remain in solution as *free* acid. It follows that if the presence of alkaline acetates, tartrates, etc., in the vinegar can be proved, mineral acids in appreciable quantities cannot have been added to the vinegar.

It has already been pointed out (see determination of acid

in baking powders, p. 137) that the alkaline salts of organic acids yield alkaline carbonates on ignition, so that if such salts are present the vinegar will yield an ash which is sensibly alkaline in reaction. Whereas if such salts have been converted to chlorides or sulphates by the action of mineral acids the ash will have a neutral reaction. Hence, if the aqueous extract of the ash gives an alkaline reaction, the vinegar does not contain mineral acid, or not more than a trace of such acid.

The same principles are also applied in the method used for the quantitative determination of the amount of mineral acid present. The volume of decinormal sodium hydroxide solution required to neutralise a measured volume of vinegar is first determined. This part of the process may, if desired, be combined with the determination of total acidity (see p. 174).

The neutral solution is then evaporated to dryness and ignited. The acetic acid in the vinegar is thus first converted into sodium acetate, and then to an equivalent amount of sodium carbonate (see equations below).

The ash is extracted with hot water, and the extract titrated with decinormal acid, using methyl orange as indicator.

If the acid in the vinegar was originally present entirely in the form of organic acid, then the volume of decinormal acid required for the neutralisation of the alkaline carbonate in the ash will be equal to the volume of decinormal alkali used to neutralise the vinegar (see, however, *Note*, p. 178).

On the other hand, if mineral acid is present the volume of decinormal acid required for the neutralisation of the ash will be less than the volume of decinormal alkali used for the neutralisation of the vinegar. The difference between these two volumes will give, in cubic centimetres of decinormal acid, the amount of mineral acid present. In illustration, equations are given below to represent the reactions taking place when (*a*) a solution of acetic acid, (*b*) a solution containing acetic and hydrochloric acid with a total acidity equal to that of (*a*) is (1) neutralised, (2) ignited, and (3) the residue titrated with acid:—

(*a*) $4CH_3COOH + \underline{4NaOH} \rightarrow 4CH_3COONa + 4H_2O$. (1)

$4CH_3COONa$ on ignition yields $2Na_2CO_3$ . . (2)

$2Na_2CO_3 + \underline{2H_2SO_4} \rightarrow 2Na_2SO_4 + 2H_2O + 2CO_2$. (3)

Thus 4 gram molecules, or 4 *equivalents*, of sodium hydroxide are used for the first neutralisation, and 2 gram molecules, or 4 *equivalents*, of sulphuric acid are used for the second neutral-

(*b*) $2CH_3COOH + 2HCl + \underline{4NaOH} \rightarrow 2CH_3COONa + 2NaCl + 4H_2O$ (1)

$2CH_3COONa + 2NaCl$ on ignition yields $Na_2CO_3 + 2NaCl$ (2)

$Na_2CO_3 + 2NaCl + \underline{H_2SO_4} \rightarrow Na_2SO_4 + H_2O + CO_2 + 2NaCl$ (3)

isation. 4 gram molecules, or 4 *equivalents*, of sodium hydroxide are used for the first neutralisation, and 1 gram molecule, or 2 *equivalents*, of sulphuric acid are used for the second neutralisation. The difference, 2 *equivalents*, represents the proportion of hydrochloric acid in the original solution.

Note.—It should be noted that it is here assumed that the alkaline carbonate obtained on ignition is produced entirely from the salts formed by the neutralisation of the free organic acid in the vinegar. As already stated, the vinegar, if free from mineral acid, normally contains small quantities of the alkaline salts of organic acids, and these also yield alkaline carbonate on ignition. The proportion of these salts is, however, so small in comparison with the total amount of free acetic acid that the amount of alkali they produce will not appreciably increase the volume of acid required for the neutralisation of the residue obtained on ignition.

Method of Carrying out the Determination of Free Mineral Acid.

This determination should only be carried out if the ash of the vinegar fails to give an alkaline reaction. The neutral solution obtained in the determination of the total acidity may be evaporated to dryness, ignited, and the residue titrated with decinormal acid (see determination of tartaric acid in baking powder, p. 137). More accurate results can be obtained by working on larger quantities. 20 or 50 c.c. of the vinegar should be neutralised with semi-normal sodium hydroxide solution, and the neutral solution then evaporated to dryness, ignited, and the residue titrated with semi-normal acid.

Colour Reactions for the Detection of Mineral Acid in Vinegar. P_H Value.

Acetic acid, being a weak acid, is only slightly ionised in aqueous solution; whereas the mineral acids, e.g. sulphuric and hydrochloric acid, which may be added as adulterants, are highly ionised.

Thus the addition of mineral acids to vinegar will tend to increase the concentration of hydrogen ions in the solution, and this difference in the hydrogen ion concentration can be made use of in detecting the presence of mineral acids in vinegar.

During recent years methods have been developed whereby it is possible to determine the concentration of hydrogen ions in a solution, or what may be called the *actual* acidity as distinct from the *total available* acidity, and it will be necessary here to introduce some explanation of the principles involved in making such determinations.

Hydrogen Ion Concentration: P_H Value of a Solution.

By the theory of ionic dissociation, an acid, when dissolved in water, does not exist entirely as molecules, but some of the molecules are dissociated into (1) positively charged hydrogen ions, and (2) negatively charged radicles. The acid properties of the solution are entirely due to the hydrogen ions. Therefore the "reaction" of a solution can only be satisfactorily expressed in terms of the concentration of the hydrogen ions. Acids differ markedly in the extent to which they become dissociated in solution, "strong" acids being highly dissociated, while "weak" acids are feebly dissociated. Pure water, the standard of neutrality, is itself slightly ionised into hydrogen ions (H^+) and hydroxyl ions (OH^-). Accurate measurements have shown that 1 water molecule in 500 million is dissociated into its ions. In other words, the concentration of hydrogen ions in water approximates very closely to 10^{-7} gms. per litre. This result is arrived at in the following manner:—

1 molecule of water in 500,000,000 is dissociated.
Hence 18 gms. ,, 500,000,000 × 18 gms. are dissociated.
,, ,, ,, $500 \times 10^6 \times 18$,, ,, ,,
That is, 1 gm. of hydrogen ion and 17 gms. of hydroxyl ion

are present in 9000×10^6 gms. of water; or approximately 1 gm. of hydrogen ion in 1000×10^7 gms. of water. Thus 1 litre of water contains 10^{-7} gms. of hydrogen ions. Since each molecule of water on dissociation gives rise to one hydrogen ion and one hydroxyl ion, the concentration of the hydroxyl ions in water is also 10^{-7} gm.-ions per litre, i.e. the concentration of both hydrogen and hydroxyl ions is 10^{-7} normal.

The product of the two concentrations is consequently 10^{-14}.

According to the laws of chemical equilibrium, this product will be constant for every solution which contains water, and thus for all aqueous solutions irrespective of the amount of hydrogen and hydroxyl ions added from other sources.

This may be expressed by the equation—

$$[H^+] \times [OH^-] = 10^{-14},$$

the square brackets being used to indicate "concentration in gram-ions per litre." In a *normal* solution of an acid, which is completely dissociated, the hydrogen ion concentration would be 1 or 10^0. By substitution in the above equation, the hydroxyl ion concentration must be 10^{-14} in order that the product should remain 10^{-14}. It follows also that solutions which have—

(1) $[H] = 10^{-7}$ and $[OH] = 10^{-7}$ are neutral.
(2) $[H]$ *greater* than 10^{-7} ,, ,, *less* than 10^{-7} are acid.
(3) $[H]$ *less* than 10^{-7} ,, ,, *greater* than 10^{-7} are alkaline.

Sörensen introduced the symbol P_H to denote the *hydrogen ion exponent.* P_H is therefore the logarithm to the base 10 of the hydrogen ion concentration in gram-ions per litre, neglecting the negative sign.

Thus P_H for distilled water = 7.
,, P_H ,, acid solution is *less* than 7.
And P_H ,, alkaline solution is *greater* than 7.

The hydroxyl ion concentration, or P_{OH}, is obtained by subtracting the P_H value from 14, but it is usual to express the reactions of all solutions, whether acid or alkaline, in terms of P_H.

In using the symbol P_H the following points should be borne in mind :—

(1) The *higher* the value of P_H the *lower* is the hydrogen ion concentration.

(2) If the P_H is altered by *one* integer the hydrogen ion concentration is increased or decreased *tenfold*.

(3) Solutions having P_H value of less than seven are acid, and those having a P_H value greater than seven are alkaline.

Hydrogen Ion Concentration and Total Acidity.

It is important to distinguish between the *hydrogen ion concentration*, or P_H value, of a solution and the *amount* of acid or alkali present. The latter can be determined by titration, but not the former. For example, it is known from the electrical conductivities of the solutions that decinormal hydrochloric acid has a hydrogen ion concentration about sixty times greater than that of decinormal acetic acid. Yet if titrated against decinormal alkali, equal volumes of the two acid solutions will be neutralised by the same volume of alkali.

The explanation of this is, that whatever the initial dissociation of the acid may be, on the addition of a base the hydroxyl ions of the base will unite with hydrogen ions present to form molecules of water.

Equilibrium is thus disturbed, and in an attempt to replace the removed hydrogen ions other molecules of the acid become dissociated. This fresh supply of hydrogen ions is in turn removed by the base, and so the process continues, until, when neutrality is reached, all the acid originally present has been ionised and the hydrogen ions removed. Even when the solution is neutral, however, the concentration of hydrogen ions, as previously explained, is still 10^{-7}.

Methods of Determining P_H.

There are two general methods which may be used for the determination of the hydrogen ion concentration of a solution.

(*a*) *The Hydrogen Electrode or Electrometric Method.*—This is the most accurate method, but it involves careful measurements of the difference in electrical potential set up between the solution and a hydrogen electrode. For this, expensive and somewhat complicated apparatus is required. Details of the apparatus and method of use are described in text-books dealing with practical physical chemistry. A simple apparatus

which may be used in connection with food analysis is described by Monier Willaims ("Analyst," 1921, 46, 315).

(*b*) *The Colorimetric or Indicator Method.*—This method, though sufficiently accurate for ordinary purposes, is not capable of such great accuracy as method (*a*).

It may be used for turbid (e.g. milk) and slightly coloured solutions, but is inapplicable to very dark solutions.

No special apparatus is required, but it is necessary to have :—

(1) A complete series of indicators which give colour changes over a wide range of P_H values.

(2) A series of standard solutions of known and constant hydrogen ion concentration.

(1) *Indicators.*—An indicator is a substance whose colour is affected by the P_H of a solution. For example, phenolphthalein is colourless in solutions which have a P_H value of less than 8. At about $P_H 8{\cdot}3$ it begins to turn pink, and the depth of colour increases with the P_H value up to about $P_H 10$, when no further change in colour is observed.

Thus phenolphthalein is an indicator which changes in colour over a range $P_H 8{\cdot}3$ to 10, and the P_H of any solution which gives with this indicator a pink colour, which is less pronounced than the extreme shade, must be somewhere within this range. It is now possible to obtain a series of indicators which show distinct and permanent colour changes over a range $P_H 1$ to $P_H 11$. The range over which the colour change takes place with each individual indicator is usually quite small. A solution of unknown hydrogen ion concentration can be tested with various indicators until one is found which changes in colour, and gives a shade intermediate between its extreme shades. The P_H of the solution must then lie somewhere within the P_H range of the indicator. For some purposes it may only be necessary to fix the P_H value of the solution between two limits in this manner.

To determine the P_H value with more exactitude, the shade of colour which the solution gives with the indicator is matched against that given by solutions of known hydrogen concentration with the same indicator. The P_H of the solution will be equal to that of the standard solution which gives the same shade of colour.

(2) *Standard Solutions of known Hydrogen Ion Concentration.*—The concentration of hydrogen ions in a solution is ordinarily greatly affected by the addition of even small

quantities of alkalis and acids, e.g. the effect of soluble alkali from glass vessels, or of the carbon dioxide of the air is appreciable. Hence it is difficult unless certain precautions are taken to keep a solution at a definite P_H value for any length of time. If, however, certain substances which exert a "buffer" action are introduced, the P_H value will remain practically unaffected by small additions of acid or alkali.

The salts of weak acids, e.g. phosphate, citrate, acetate, etc., exert a "buffer" action as the following considerations show. The alkali *salts* of weak acids are highly ionised, thus a solution of sodium acetate, for example, will contain sodium ions and acetate ions (CH_3COO^-). If now a small quantity of hydrochloric acid be added, the hydrogen ions must come into contact with the acetate ions and practically all go to form undissociated acetic acid. For since acetic acid is a weak acid, hydrogen ions and acetate ions can only exist together in water to a very limited extent. The effect of the added hydrochloric acid on the concentration of the hydrogen ions is thus reduced to a minimum—

$$(CH_3COO)^- + Na^+ + H^+ + Cl^- \rightarrow \underset{\text{(undissociated)}}{CH_3COOH} + Na^+ + Cl^-$$

A series of solutions having constant P_H values ranging from 1·2 to 9·2 can be prepared by mixing standard solutions of such salts as sodium citrate, sodium phosphate, and potassium dihydrogen phosphate in varying proportions with standard solutions of acid and alkali. For accurate work it is advisable that the P_H of some, at least, of these solutions should be checked by the electrometric method, and the others can then be standardised accordingly. If the solution to be tested is itself coloured or turbid, it is possible by carrying out the matching in a *comparator* to compensate for the colour or turbidity of the original solution.

For details of the method, see Evers, "Analyst," 1921, **46**, 393.

Detection of Mineral Acid on Vinegar by P_H Value.

The use of methyl violet for detecting mineral acid in vinegar was suggested by Hilger as far back as 1876. When a few drops of a 0·1 per cent. aqueous solution of methyl violet are added to untreated vinegar, no colour change is observed. In the presence of as little as 0·2 per cent. of free

mineral acid the colour becomes blue, with 0·5 per cent. blue-green, and with 1 per cent. green. These changes in colour are to be attributed to the increase in the hydrogen ion concentration of the solution, which results from the addition of the mineral acid, since methyl violet shows the following range of colour changes :—

P_H3 → violet ; P_H2 → blue violet ; P_H1 → green-blue.

Recently other observers (Kling, Lassieur, and Lassieur, "Compt. Rend.," 1922, **174**, 165 ; and "Ann. Chim. analyt.," 1922, **4**, 135) have shown that hydrogen ion concentration of vinegar containing from 5 to 7 per cent. of acetic acid varies from $P_H2·5$ to $P_H2·8$, and that the addition of 0·24 per cent. of sulphuric acid causes this value to become $P_H1·48$ to 2·02.

Measurements have been made electrometrically and also colorimetrically, using the indicator thymol blue (thymol sulphophthalein), which has colour range from $P_H1·2$ to 2·8 (red to yellow).

Alcohol in Vinegar.

Owing to its method of preparation, vinegar may contain small quantities of alcohol which have escaped oxidation.

If present in appreciable quantity, the amount can be determined after neutralising the solution by the method described under "Alcoholic Beverages" (see p. 197). A qualitative test can be made in the following manner: Distil 50 to 100 c.c. of the liquid, neutralise the distillate with sodium hydroxide, and test for alcohol by the iodoform test.

Iodoform Test.—Warm the solution with a strong solution of iodine in potassium iodide. Add sodium hydroxide solution until the liquid is nearly decolorised. On standing, a yellow precipitate with the characteristic odour of iodoform is obtained if alcohol was present.

FRUIT JUICES AND VEGETABLE ACIDS.

The juices expressed from certain fruits, e.g. grapes, apples, lemons, and limes, are used as beverages, and also as flavourings for jellies, creams, etc. (see also Fruit Essences, p. 254).

Grape juice is used almost entirely in the fermented state.

In this form it will contain considerable quantities of alcohol, and is therefore classified as an alcoholic beverage (see p. 196).

The acid present is chiefly tartaric acid and acid tartrates (compare wine vinegar, p. 172).

Apple Juice.—Cider is the expressed juice of the apple, and when fresh, and before fermentation has set in, it is known as *sweet cider.*

On keeping, alcohol is formed by the action of a yeast, which is found in considerable quantity on the outside of the apple, as well as in the soil in which the tree grows.

Cider usually contains from 3 to 6 per cent. by volume of alcohol, and malic acid is present.

Lime Juice and Lemon Juice.—The juice of both the lime and the lemon are known commercially as lime juice. A genuine unadulterated lime juice should give about 8 per cent. of total solids and an acidity of about 7 per cent. calculated as citric acid, in which form the acid is chiefly present. Many of the preparations sold as *lime juice, lemon squash*, etc., are artificially prepared mixtures containing organic acids, e.g. citric and tartaric acid, sugar, colouring matter, and preservatives. Such preparations also sometimes contain small quantities of mineral acids, including phosphoric acid.

Examination of Lime Juice, Lemon Squash, etc.

The *total solids* and *acidity* may be determined as described under vinegar (see p. 173). In this case, however, the acidity should be calculated as *citric acid* and not as acetic acid (1 c.c. N/10 alkali = 0·0064 gm. citric acid).

Nature of the Acid.—Tartaric acid should not be present in preparations which are alleged to have been made from limes or lemons, and citric acid should be the only acid present.

Tartaric acid and cream of tartar should be tested for in the residue obtained on evaporation as described under Vinegar (p .174).

Portions of the solution, concentrated if necessary, may also be tested for sulphuric acid, hydrochloric acid, and phosphoric acid by the usual qualitative tests. In these preparations the salts of these acids are unlikely to be present in sufficient quantities to invalidate the tests (compare Vinegar, p. 176).

If sulphuric or hydrochloric acid is present, the amount

can be determined by the method described for the determination of mineral acids in vinegar, p. 176.

Colouring matter, if added, is usually in such small quantities that it is difficult to prove its presence. But it may be noted that lime juice and lemon juice are not yellow in colour, and that in a general way it is quite impossible to get the deep colours of these so-called fruit juices from the fruit alone, i.e. without the addition of some colouring matter.

It is sometimes possible to show the presence of a dye by boiling a piece of white woollen cloth for some time in a concentrated solution of a sample (see p. 146).

Detection of Alcohol.—Small quantities of alcohol are sometimes present, and can be detected in the manner described under vinegar.

Preservatives.

Any of the usual preservatives, e.g. boric acid, salicylic acid, benzoic acid, and sulphites, may be found, but it is probable that in future the addition of only one or other of the last two named will be allowed (see p. 202).

Boric Acid.—Acidify the solution with hydrochloric acid and test with turmeric (see p. 21).

Sulphites.—To a portion of the solution contained in a small conical flask add dilute sulphuric acid and a small piece of granulated zinc. Loosely cork the flask with a cork covered with a piece of filter paper moistened with lead acetate solution. (Compare Gutzeit's Test, p. 217.) Any sulphite present will be reduced to sulphide, and the paper will be stained dark brown by the formation of lead sulphide.

Salicylic Acid and Benzoic Acid.—Salicylic and benzoic acids can be extracted from aqueous solutions containing the free acids by ether (or chloroform).

The usual qualitative tests for these acids can be applied to the residue obtained after removal of the solvent by evaporation at a low temperature (usually room temperature). Instead of removing the ether by evaporation the acid may be extracted from the ethereal solution by shaking with a dilute solution of ammonia. The ammoniacal solution, which will contain the ammonium salts of the acids, can, after evaporation almost to dryness, be examined for the presence of salicylate and benzoate.

On the addition of a few drops of neutral ferric chloride solution * to the ether residue, or concentrated ammoniacal solution, a deep purple colour is observed in the presence of salicylic acid, and a buff coloured precipitate of ferric benzoate in the presence of benzoic acid.

For methods of determining the amounts of benzoic and salicylic acids, when these substances are used as preservatives, see "Food Inspection and Analysis" by Leach.

* Add dilute ammonia to a solution of ferric chloride until a distinct precipitate begins to form. Filter off the precipitate and use the filtrate.

Chapter VII.

BEVERAGES.

TEA, COFFEE, AND COCOA.

These substances, which are of seed or leaf origin, and which are used in the production of beverages, need only be briefly dealt with here. Apart from their stimulating action their value to the consumer is largely dependent on the presence of small amounts of characteristic flavourings and aromas. The chemical analysis of such products yields little, beyond the detection of adulteration, which is of assistance in forming any judgment as to the relative merits of different samples, and this is a matter which must be left to the expert taster to decide.

Further, the methods usually employed for the determination of some of the more important constituents of these substances, e.g. caffeine and tannin, yield reliable results only when considerable practical experience of the various processes involved has been obtained, and on this account descriptions of these methods have been omitted here. The microscopical examination often furnishes information which cannot be obtained by chemical methods, but here also the observer needs to have some training in detecting characteristic features, and must be familiar with the structure of the material under examination.

Instruction on this subject can readily be given by means of practical demonstrations with a microscope, but written instructions, even when suitable illustrations are provided, usually prove unsatisfactory substitutes for such demonstrations, and are likely to be intelligible only to those who have some previous knowledge of the subject. The microscopy of these substances is dealt with fully in "A Compendium of Food Microscopy," Clayton (Baillière, Tindall & Cox), and "Microscopy of Vegetable Products," Winton (Wiley & Son).

Tea.

Tea is the dried and prepared leaf of a shrub, *Camellia Thea*. The differences in the many kinds of tea known in commerce depend not on botanical distinctions, but on the age of the plant at the time the leaf is gathered, the position of the leaf on the shoot, and the methods used for drying and preparing the leaves. Thus the choicest China tea is prepared from the youngest, or end, leaves of the shoots of young plants which are scarcely more than buds. Whilst the old leaves of old plants are used for the production of inferior teas.

Teas are also divided into two classes, "green tea" and "black tea," according to the method of preparation used.

Green tea is prepared by drying the fresh green leaves after they have been rolled to express the juice.

In preparing *black tea* the leaves are first withered by exposure to sun and air, so that changes due to oxidation and fermentation take place, and the leaves are subsequently dried.

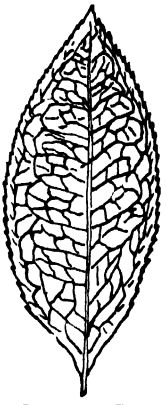
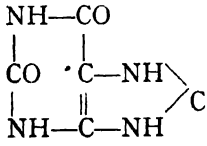


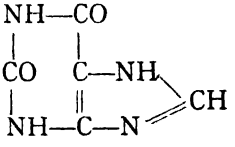
Fig. 17.—Tea Leaf.

If leaves of tea which have been softened by soaking for a few minutes in hot water are examined under a microscope, it will be seen that the leaf is oval in shape and has a well marked serrated edge (see Fig. 17). To the underside of the leaf are attached hair-like threads with club-shaped ends. These characteristics form a means of distinguishing tea from other leaves.

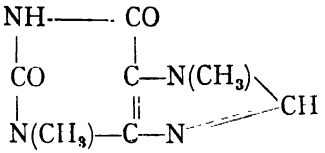
The stimulating properties of a tea infusion are due to the presence of caffeine, or theine (trimethyl-xanthine), which is a compound of the uric acid series. The formula of uric acid and those of some of its important derivatives are given below.



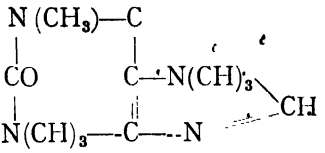
Uric acid
(2 : 6 : 8-trioxy-purine).



Xanthine
(2 : 6-dioxy-purine).



Theobromine
(3 : 7-dimethyl-xanthine).



Caffeine or theine
(1 : 3 : 7-trimethyl-xanthine).

The percentage of caffeine in tea varies from 2·5 to 4 per cent. In cocoa, dimethyl-xanthine or theobromine is present as well as caffeine.

Tannin or tannic acid is the constituent which gives the tea its characteristic astringent taste. Tannin is a complex substance which is derived from gallic acid or trihydroxy benzoic acid, $C_6H_2(OH)_3COOH$, and it yields this substance on boiling with dilute acids.

A number of different processes for the estimation of tannin have been devised, but none can be said to yield absolutely accurate results. In giving the results of tannin estimations it is necessary, for the purpose of comparing the results with those of other observers, to state the method used for determining the tannin. The difficulty of obtaining accurate results is to be attributed largely to the fact that the exact composition of tannin has not yet been settled, and also that the tannins obtained from different sources are probably not identical in constitution. . The tannin present in genuine tea leaf usually varies from about 9 to 18 per cent., and averages about 12 per cent. If the leaves of some other plant, or tea leaves which have been previously exhausted with water and then dried, have been added to the tea, the tannin content will be low, whilst excess of tannin indicates the presence of added astringent matter, e.g. catechu. It has already been noted (see Vol. I., Chap. IV.) that pure silk readily absorbs tannic acid, and Vignon (*Compt. rend.*, 1898, 127, 369) has suggested a simple method for the approximate determination of tannin in a tea infusion which is based on this reaction.

An extract is prepared which contains about 0·1 per cent. of tannin (1 gm. of tea to 150 c.c. of water). The total solid matter is determined in a portion of the extract. 5 gms. of pure white silk, free from dressing, are introduced into another portion of the extract which is kept at 50° C., and occasionally shaken for two hours. The silk absorbs the tannin, and the

difference between the total solids in the extract before and after treatment with the silk is returned as tannin.

The assumption is here made that silk will completely absorb the tannic acid from solution, and will not absorb other substances which may be present, e.g. gallic acid and dextrose. It would appear, however, that silk does not absorb all tannin materials in the same proportion, and the extent to which the tannin is absorbed will be dependent, in some degree, on the form in which the tannin is present. Under some conditions also the silk will absorb gallic acid.

Thus the results obtained by this method should be regarded as giving only an approximate measure of the amount of tannin present.

Adulteration of Tea.

The greater proportion of the tea now solid is genuine. Substitution of foreign leaves and other forms of adulteration once commonly practised are now, owing to a system of careful inspection, extremely rare. Fraud may, however, be practised by the substitution of inferior grades of tea for those of good quality, and this in many cases is beyond the power of the analyst to discover. Adulteration may take the form, as already stated, of the addition of foreign leaves, or of tea leaves which have been exhausted with water and then dried. Astringent matters, e.g. catechu and gambier, have been used to increase the tannin content. Materials are also occasionally added as *facing*, i.e. to improve the colour and appearance of the leaf. These include Prussian blue, indigo, gypsum, plumbago, turmeric, and a number of other bodies; whilst small proportions of alkaline salts, e.g. sodium bicarbonate and borax, may be used with a view to producing a darker extract when tea is infused with water (see p. 192).

Tea Infusions.

The infusion of tea as ordinarily prepared for making a beverage, by pouring *boiling* water on to the leaves, does not contain the whole of the soluble constituents of the leaf, since the conditions of extraction are not such as entirely to exhaust the tea. It is, of course, well known that the vessel in which the tea leaves are placed should previously be heated, otherwise the water poured on to the tea is cooled very considerably, and an extract lacking in flavour is obtained.

The essential oil and a large proportion of the caffeine are usually extracted by infusing for about five minutes, but the tannin dissolves more slowly, and the amount extracted will depend on the duration of the infusion.

When a tea infusion or an aqueous solution of tannic acid is added to a solution of gelatine, the gelatine is precipitated. It is to this reaction with gelatine and other protein bodies that the injurious effects of excessive tea drinking are to be mainly attributed.

Excess of tannin in a tea infusion can best be avoided by allowing the tea to infuse for not more than five minutes, and if the infusion is then poured off the leaves, further extraction of the tannin is prevented. As stated above, this extract will contain the essential oil and most of the caffeine, on which the stimulating effect of the tea depends.

The addition of alkali to the water used for preparing the infusion makes the extract darker in colour, but this alkaline extract does not precipitate gelatine. Also, if alkali is added to an ordinary tea infusion, the colour of the infusion darkens, and the solution will no longer precipitate gelatine.

This darkening and change in behaviour is due to the oxidation of the tannin in alkaline solutions, and the same changes are observed when alkali is added to an aqueous solution of tannic acid.

The amount of tannin extracted from tea by boiling water, if the duration of infusion is kept constant, is dependent on the nature of the tea, and also on the character of the water.

H. L. Smith ("Pharmaceutical Journal," June, 1913), determined the amounts of tannin extracted from a number of different samples of Indian and China teas by distilled water, waters having temporary and permanent hardness, and water containing sodium bicarbonate. The results showed that distinctly less tannin was extracted by hard waters than by distilled water, whilst the amount of caffeine extracted was unaffected by the character of the water. It would appear that tannin of China teas is not so readily extracted as that of Indian and Ceylon teas. For example, after ten minutes infusion about 89 per cent. of the tannin was extracted in the case of a Ceylon tea, and only 66 per cent. of the tannin in the case of a China tea, and it is suggested that in the China tea the tannin is present in a less soluble form.

The addition of sodium bicarbonate increased the amount

of tannin extracted, although, as already stated, these alkaline infusions do not precipitate gelatine.

Infusions prepared with London tap-water were, owing to the alkalinity of the water, slightly darker in colour than those prepared with distilled water, but the alkalinity was insufficient to prevent the precipitation of gelatine. In spite of the darker colour of the extract, the tap-water infusions contained less tannin than those prepared with distilled water, and it is necessary to point out that the colour of the infusions gives no reliable indication as to the amount of tannin which is present in the extract.

Coffee.

The coffee bean forms the seed (kernel) of a fruit which is not unlike the cherry in appearance. The fruit has two divisions each containing a single seed or bean, which is surrounded by a tough parchment-like membrane. In order to obtain the coffee bean itself, the pulp or outer portion is first removed, and the beans are then "hulled" to remove their outer coating. The coffee berries are roasted before use, and although the raw berries can be purchased, the greater part of the coffee sold in commerce consists either of the roasted "beans," as the berries are usually termed, or of the same ground to a coarse powder. Coffee in the ground form can be more readily adulterated than the beans or berries, although even in the latter case attempts have been made to adulterate the coffee by mixing in pellets, prepared usually from roasted cereals, which have been moulded in imitation of the coffee berry.

Constituents of Coffee.—The chief constituents of coffee are caffeine, a mixture of acids known as caffetannic acid, considerable proportions of carbohydrates (in the form of cellulose and sugars), fat, essential oil, and aromatic substances. During roasting the sugars are partly caramelised, flavour is developed, and the bean becomes less tough, and can be more easily ground.

Adulteration of Coffee with Chicory.

The principal adulterant of ground coffee is chicory, i.e. the roasted root of the chicory plant. So-called "French Coffee" is usually a mixture of coffee, burnt sugar, and chicory.

The best method of detecting chicory is by means of the microscope, although the chemical analysis also yields results which are of some value in this respect. For example, chicory only contains a small proportion of fat (1 to 2 per cent.) whereas coffee contains from 10 to 14 per cent. of fat, and both the total solids in the aqueous extract and the ash are higher in the case of chicory than in that of coffee.

A careful examination of the coarsely crushed grains of the ground sample, when spread out on white paper, will often serve to detect the particles of chicory, which are apparent from their dark and gummy appearance. When crushed between the teeth their soft consistency and characteristic flavour are very distinctive.

A simple test for chicory can also be made by shaking some of the ground material into water, or into a saturated solution of common salt.

Coffee, on account of its fat content, floats on the surface of the liquid; whilst chicory, and also other adulterants, such as cereals and mineral ingredients, sink. The grains of chicory, as they fall through the liquid, produce reddish-brown streaks in the liquid, which gradually becomes coloured. This difference may be demonstrated in the following manner: Take three beakers, each containing an approximately equal volume of a saturated solution of common salt.

Shake from a test tube into (1) pure ground coffee; (2) ground roasted chicory; (3) a mixture of (1) and (2). Care should be taken that the amount of powder, added to the salt solution, is approximately the same in each case. In (1) nearly all the material will float on the surface, and the liquid will be only slightly coloured; whilst in (2) a sediment will rapidly be formed and the liquid will become dark yellow-brown in colour. The behaviour of (3) indicates that it is a mixture of (1) and (2).

Cocoa and Chocolate.

The various preparations sold as cocoa and chocolate are prepared from the seed, or bean, of the tree *Theobroma cacao*. The beans, which are about the size of almonds, grow closely packed together in pods.

After removal from the pod, the beans, which when fresh are white in colour, are allowed to undergo a process of fermentation, and the flavour of the seed depends largely on the

careful regulation of this process. The seeds are next dried in the sun until they assume their characteristic red-brown appearance.

For the production of chocolate and cocoa the beans are cleaned and carefully roasted, the flavour being thereby developed and the thin shell which surrounds the seed loosened. The roasted seeds are crushed, and the shells separated from the seed proper by a winnowing process.

Infusions can be prepared from the shells which have a taste and flavour resembling that of cocoa, and crushed cocoa shells are sometimes sold at a low price for this purpose.

The crushed fragments of seed, or kernel, are called *cocoa nibs*, and for the preparation of chocolate they are finely ground into a paste. If *plain* or *unsweetened* chocolate is required, this paste is run directly into moulds, but for making sweet chocolate the nibs are first mixed with sugar and vanilla or other flavouring agents before being moulded.

In making cocoa a portion of the oil or fat, known as *cocoa butter*, is removed by pressing the ground seeds between heated plates, and the pressed mass is then reduced to a fine powder, either directly or after treatment with ammonia or other alkali to render the product more soluble in water (see below). It is held that the large amount of fat, about 40 to 54 per cent., contained in cocoa seeds is difficult of digestion to many, and hence the desirability of removing a portion of the fat.

Constituents of Cocoa.—The chief constituents of the cocoa nib are fat, starch, mineral matter, proteins, and other nitrogenous bodies, of which theobromine is the most important, a small proportion of caffeine being also present.

Adulteration of Cocoa.

The proportion of water-soluble matter in cocoa is low, usually about 20 to 25 per cent. of the total solid matter present, and in preparing the beverage it is desirable that the emulsion obtained shall be as perfect as possible.

This can be effected by grinding the cocoa to a very finely divided powder, but the addition of alkali is sometimes resorted to, with a view to facilitating the emulsification of the fat.

The use of alkali is usually detected by an abnormally high ash, and by the increased alkalinity of the ash. This

alkalinity is expressed as the number of cubic centimeters of decinormal acid required to neutralise the ash from 1 gm. of the sample. In untreated cocoa the ash rarely exceeds 5·5 per cent., and the alkalinity is usually not more than 3·8; whilst in cocoa treated with alkali the ash sometimes reaches 8·5 per cent., and the alkalinity may be as high as 6 or even 8.

The removal of fat, the addition of sugar beyond certain prescribed limits, and the addition of other forms of fat, and also of starch, are regarded as forms of adulteration; unless the nature of the product is clearly stated on the label.

For further information on this subject, reference should be made to "Cocoa and Chocolate," Knapp (Chapman & Hall).

ALCOHOLIC BEVERAGES.

Introduction.—This subject will be dealt with very briefly. Alcoholic beverages may be divided into four classes, as follows:—

(*a*) Fermented fruit juices—wines, cider, perry.
(*b*) Malt liquors—beer, stout.
(*c*) Distilled liquors (spirits)—whisky, brandy, gin, rum.
(*d*) Liqueurs.

(*a*) In the case of liquids of the first class, the alcohol is produced by the fermentation of the sugar in the fruit juice. This fermentation proceeds until the sugar (or protein) is exhausted, or the yeast growth is checked, owing to the inhibitory action of the alcohol when its content reaches approximately 18 per cent. by volume. Hock and claret belong to this class.

Fortified wines are those of this class to which additional alcohol has been added, e.g. port and sherry. If the alcohol is added before all the sugar is exhausted, fermentation is checked, owing to the concentration of the alcohol, and the resulting wine is sweet.

In the production of sparkling wines, part of the fermentation takes place after bottling.

(*b*) In the production of liquids of the second class, the starch in grains, such as barley, is first converted into the sugar maltose by the action of the enzyme diastase, produced when the barley is allowed to germinate (production of malt, see p. 104). The maltose is then extracted by water, and the solution boiled with hops. After cooling, the maltose is converted first to glucose and then to alcohol and carbon dioxide by the action of the enzymes maltase and zymase in

the yeast which is added (see pp. 105 and 106). In the production of stout, malt which has been roasted is employed.

Glucose is sometimes employed in connection with the manufacture of beer, and reference should be made to the possibility of contamination by arsenic by this means (see pp. 111 and 216).

(*c*) The liquids in the third class (spirits) are distillates, and are thus characterised by high alcohol content and low total solids. Whisky is obtained by distillation of a dilute solution of alcohol obtained from grain, as in the production of beer; brandy by distillation of fermented grape juice; rum by distillation of fermented molasses, and gin by distillation of a solution of alcohol, obtained from grain, containing various flavouring agents, notably oil of juniper.

(*d*) In the production of liqueurs concentrated aqueous solutions of alcohol, obtained as distillates, are mixed with flavouring agents, and after standing some time are distilled. Cane sugar and colouring matter are then added to the distillates.

The percentages of alcohol by volume in various alcoholic beverages are approximately as follows: (*a*) claret and hock 8 to 12, port and sherry 15 to 24; (*b*) beer and stout 3 to 7; (*c*) brandy, whisky and gin 30 to 40; (*d*) liqueurs 35 to 55. The concentration of alcohol in beverages is, however, usually referred to in terms of *proof spirit*, which is described later.

Determination of the Percentage of Alcohol in an Alcoholic Beverage.

The operations involved (distillation, so as to remove the whole of the alcohol; and determination of the specific gravity of the distillate, from which the percentage of alcohol is obtained) are described in Volume I, page 249. For practice in the determination, the percentage of alcohol in beer should be carried out.

Obtain two large beakers, and in one place about 300 c.c. of beer, and pour it backwards and forwards from one beaker to another until it ceases to froth. This operation is to allow some of the carbon dioxide dissolved in the liquid to escape, otherwise when the liquid is distilled considerable frothing will take place.

Measure out 250 c.c. of the beer which has been treated in this way, and put it into a flask of about 750 c.c. capacity.

Add a small quantity of porous tile to facilitate regular boiling when the liquid is distilled. Attach a water condenser to the flask, and use a 250 c.c. flask as receiver. Cotton wool should be packed loosely between the neck of the receiving flask and the end of the condenser.

Heat the flask gently over a wire gauze, using only a very small flame, until all tendency to froth has ceased. Continue the distillation until about *two-thirds* of the liquid has passed over, by which time all the alcohol contained in the beer will be in the distillate.

Dilute the distillate with distilled water until its volume is 250 c.c. at 60° F. (15·5° C.). Determine the specific gravity of the diluted distillate (after well mixing by shaking) by means of the specific gravity balance.

From the alcohol table ("Quantitative Analysis," by Clowes and Coleman) find the percentage of alcohol by volume in the diluted distillate. Since the distillate, containing all the alcohol in the original beer, was diluted, so that its volume was equal to that of the beer taken for the experiment, the percentage by volume of alcohol in this diluted distillate is the percentage of alcohol, by volume, in the original beer.

The excise duty on beer is not calculated directly on its alcohol content, but on the specific gravity of the solution (the wort) from which the beer was made by fermentation. This specific gravity is referred to as the "original gravity of the wort," the specific gravity of water being taken as 1000. In order to obtain this original gravity in a beer ready for use, it is necessary to determine the specific gravity of the liquid left in the distilling flask in the alcohol determination, after dilution to the original volume of the beer. The acidity of the beer, which is partly due to oxidation of alcohol, must also be determined. From the results obtained, and by reference to tables, the "original gravity" is obtained by means of a calculation which is somewhat complex.

Proof Spirit.

In this country the excise duty on most solutions of alcohol is based on a mixture of alcohol and water of a definite concentration, known as "proof spirit." This is an aqueous solution of alcohol which contains 49·3 per cent. by weight of alcohol, and 57·1 per cent. by volume (the specific gravity of

pure alcohol being 0·79, the percentage by volume is numerically greater than the percentage by weight).

Originally proof spirit was defined to be alcohol of such a strength that when gunpowder was moistened with it and a light applied, it was just possible to set fire to the powder. If water was in excess of that in proof spirit, the powder would not burn.

A spirit containing less than 49·3 per cent. by weight, or 57·1 per cent. by volume of alcohol, is said to be under proof (U.P.), and conversely if the percentages are greater than these it is over proof (O.P.).

A spirit 30 *degrees* under proof contains as much alcohol in 100 volumes as 70 volumes of proof spirit.

Since 100 volumes of proof spirit contain 57·1 volumes of alcohol,

$$70 \text{ volumes of proof spirit contain } \frac{57{\cdot}1 \times 70}{100} = 39{\cdot}97$$

volumes of alcohol.

Or, 100 volumes of a spirit 30° under proof contain 39·97 volumes of alcohol, or the percentage by volume of alcohol in such a spirit is 39·97.

Similarly, a spirit which is 74·5° over proof will contain in 100 volumes as much alcohol as 174·5 volumes of proof spirit.

100 volumes of proof spirit contain 57·1 volumes of alcohol.

$$\therefore 174{\cdot}5 \text{ volumes} \quad \text{,,} \quad \text{,,} \quad \frac{57{\cdot}1 \times 174{\cdot}5}{100} \quad \text{,,} \quad \text{,,}$$

$$= 99{\cdot}64.$$

That is, a spirit 74·5° over proof contains 99·64 per cent. by volume of alcohol.

Spirits sold as beverages in this country at the present time must be between 30° and 50° under proof.

The duty on alcohol at present is 74s. per gallon of proof spirit, or 72s. 6d. in the case of spirits, such as whisky, which have been three years in bond; but under certain conditions duty-free alcohol may be obtained for use in research laboratories of universities, etc. The actual cost of production of alcohol is very much less than the duty on it.

Denaturing of Alcohol.

Alcohol which is to be used as a fuel (see Vol. I., p. 248) or in operations such as the manufacture of varnish stains, etc.,

is rendered unfit for drinking by the addition of certain substances which cannot be easily removed from it, and which give to it an unpleasant odour and taste. This process is termed "denaturing," and alcohol treated in this way is known as "methylated spirit."

Various processes are employed for denaturing, but in this country crude methyl alcohol * (crude wood spirit), or a mixture of this substance and paraffin oil, are usually employed.

Crude wood spirit, one of the products of the destructive distillation of wood, contains methyl alcohol, acetone, and small quantities of substances of objectionable taste and smell. It should be noted that methyl alcohol is a poisonous substance.

Methylated spirit obtained retail at the present time contains both wood spirit and paraffin, and is coloured by the addition of a violet dye. The presence of paraffin is shown by the turbidity produced on the addition of water, as although the hydrocarbons of which the paraffin is composed are soluble in alcohol, when the alcohol is diluted with much water they separate out in a very fine state of division.

Alcohol denatured by the addition of wood spirit only is supplied under certain conditions to manufacturers and laboratories. Such alcohol is known as "Industrial Methylated Spirit." No turbidity is produced on the addition of water to this spirit.

For further information on the subject of alcoholic beverages, see "Alcohol" by Simmonds (Macmillan).

* Hence the name "methylated spirit."

Chapter VIII.

THE PRESERVATION OF FOOD; POISONOUS METALS IN FOOD.

Introduction.—For various reasons it is necessary to keep food for a considerable period under such conditions that when required it shall be in a fit state for consumption. That is, the material is kept in such a way that growth of moulds and bacteria is impossible.

The most important methods employed with this end in view are (*a*) preservation by means of cold storage; (*b*) heating the material to bring about destruction of organisms, and subsequently storing in airtight containers made of tinned iron or glass.

In other methods of preservation, for example, pickling and salting, solutions of substances such as acetic acid (vinegar), common salt, etc., are employed, or common salt and nitre (sodium or potassium nitrate) used in the solid form. Here again the conditions are unfavourable to the growth of moulds and bacteria. Cane sugar often acts as a preservative (for example, in jams, condensed milk, etc.), since moulds and bacteria do not grow readily in concentrated solutions of this substance. This may possibly be due to an osmosis effect due to the presence of the sugar.

In many cases chemical preservatives such as boric acid, borax, formaldehyde, salicylic acid, benzoic acid, sulphurous acid, etc., are employed. A *preservative* may be defined as a substance which is capable of inhibiting, retarding, or arresting the process of fermentation, acidification, or other decomposition of food, or of masking any of the evidences of any such process. It should be noted, however, that common salt, acetic acid or vinegar, nitre (sodium or potassium nitrate), sugars, alcohol, spices, essential oils, or any substance added to food during the process of curing known as "smoking" are not to be regarded as preservatives from a legal point of

view. It has apparently never been settled definitely as to whether or not the substances commonly used as preservatives are harmful when taken in small quantities. It does, however, seem possible that substances such as these, which have a germicidal action, and which, if taken in large quantities would be poisonous, may have some effect on the processes of digestion. The general opinion seems to be that sulphur dioxide (and sulphites) and benzoic acid (and benzoates) are the least harmful of the substances usually employed as preservatives. In the case of the former also, a large amount of the preservative disappears during storage and cooking, partly by volatilisation of sulphur dioxide and partly by oxidation to a harmless sulphate. An objection, however, to the use of sulphite preservatives for meat is that the putrefactive odour of decaying meat is thereby removed, so that the preservative may conceal the condition of the meat.

It is probable that in future the only two preservatives legally recognised will be sulphur dioxide (and sulphites) and benzoic acid (and benzoates), provided that no article of food may contain both. The maximum amounts proposed for different foods and beverages are as follows :—

Sulphur dioxide and sulphites (expressed as sulphur dioxide).—Sausages, 3 grains per lb. ; jam, 0·3 grain per lb. ; fruit and fruit pulp, not dried, 5 grains per lb. ; dried fruit, 7 grains per lb. ; beer and cider, 5 grains per gallon ; alcoholic wines, 3 grains per pint ; non-alcoholic wines, cordials, and fruit juices (sweetened or unsweetened), 3 grains per pint.

Benzoic acid and benzoates (expressed as benzoic acid).—Coffee extract, 3 grains per lb. ; non-alcoholic wines, cordials, and fruit juices (sweetened or unsweetened), 5 grains per pint ; sweetened mineral waters and brewed ginger beer, 1 grain per pint.

In the case of sausages, jam, and coffee extract, a statement on the label as to the nature and amount of preservative present will probably be necessary.

The examination of foods for the presence of added preservative is dealt with under the individual substances.*

Another method for the preservation of food referred to above is met with in what are termed " smoked " foods.

* See Detection of Boric Acid in Milk, Cream and Sausages, pp. 21, 26 and 165 ; Formaldehyde in Milk, p. 23 ; Sulphite in Glucose Syrup, p. 219 ; Meat, p. 166 ; Fruit Juices, p. 186 ; Salicylic and Benzoic acids in Fruit Juice, p. 186 ; Hydrogen Peroxide and Sodium Fluoride in Cream, p 26.

Bacon, for example, is often preserved in this way. The material, usually after preliminary salting, is exposed directly to the smoke from a wood fire, and becomes impregnated with small quantities of acetic acid and phenols, the latter having pronounced germicidal action.

The removal of water from a substance often facilitates its preservation. Thus the preservation of milk by the production of so-called dried milk has previously been referred to. It is, of course, well known that many fruits, after the removal of a large proportion of the water which they contain, remain unaltered for a considerable time. Moulds and bacteria are unable to flourish in the absence of moisture.

A method of preservation by what is known as gas storage may become of some importance. Partial or complete replacement of air in the storage receptacle by a gas, such as carbon dioxide or nitrogen may possibly afford a method of preservation for certain materials, especially fruit. It should be noted, however, that in this process and in others in which the material is not heated, anaerobic organisms, that is, those which can multiply in the absence of free oxygen, are not destroyed, although material stored in this way may be free from moulds.

Considering the subject of food preservation as a whole, it will be seen that the methods employed fall more or less into two groups. In the one case, as for example by the application of heat or by addition of a chemical preservative, an attempt is made to destroy organisms, and in the other, as for example in cold storage or by drying, an attempt is made to prevent their growth.

At the present time a large amount of work in connection with the preservation of food is being carried out in this country for the Food Investigation Board of the Department of Scientific and Industrial Research. These investigations are referred to in the Annual Reports of the Board, and in Special Reports published since 1918 by H.M. Stationery Office.

COLD STORAGE.

In connection with this subject it is necessary to distinguish between *chilling* and *actual freezing*.

When material such as beef or fruit is kept at a temperature of 0° C., or a little higher, it is said to be "chilled." At this temperature the water in the substance would not be frozen, as a temperature below 0° C. is necessary for the separation of ice from a solution. Beef, for example, can be kept practically unchanged in this condition for three weeks or a month, but not longer. This method could not, therefore, be employed for meat imported from Australia or New Zealand, as in these cases storage for considerably more than a month is necessary.

On the other hand, mutton, rabbits, etc., are often *frozen* and kept at a temperature well below 0° C. In this condition the frozen material can be kept for as long as three years and still be quite wholesome when "defrosted." This process, so far, has been more successful in the case of mutton than of beef, but frozen Australian beef is now being imported in small quantities.

In most cases of cold storage and refrigeration on a large scale, the cold is produced by the evaporation of a substance such as liquid carbon dioxide, sulphur dioxide, or ammonia (i.e. liquefied ammonia gas, not ammonia solution, which is sometimes erroneously called liquid ammonia). These substances have critical temperatures of 31° C., 136° C., and 157° C. respectively, and can thus be condensed to liquids by pressure alone at the ordinary temperature. By allowing these liquified gases to evaporate in a pipe surrounded by another containing a concentrated solution of common salt, heat is absorbed from the brine, which is thereby cooled considerably below 0° C. This brine then circulates through the refrigerating chambers.*

It will be obvious that the lower the temperature of the cold store, the greater will be the expense of maintenance of this temperature.

It may have been noticed that when a piece of *frozen* meat is kept at the ordinary temperature a large amount of liquid

* This cold brine is also employed in connection with the manufacture of margarine (p. 52) and of ice. In order to obtain *clear* ice the water to be frozen is agitated to allow air bubbles to escape, the clearest ice being made from freshly distilled water which contains very little gas in solution

collects on the dish on which it is placed. This is very largely moisture, which has condensed from the air on the cold surface of the meat during thawing. That is, the meat is at a temperature below the *dew-point* of the air, and consequently the air in its immediate neighbourhood is also cooled below the dew-point (see Vol. I., p. 179). Usually, however, frozen meat is "defrosted" before retail sale, and the process is carried out in *dry* air in order to prevent this deposition of moisture, which otherwise may lead to the removal of part of the extractives from the meat.

It has been shown recently that carnosine, one of the extractives of meat, disappears to a large extent during cold storage. This may account for the difference in flavour sometimes noted in imported and home-killed meat. In this connection reference should be made to a paper by W. M. Clifford, "Biochem, J.," 1922, **46**, 341.

Many considerations, such as the rate of freezing and defrosting, are of great importance in connection with frozen meat. For further information on this subject, see "The Preservation of Food by Freezing, with Special Reference to Fish and Meat," by Stiles, Food Investigation Board Report, No. 7, 1922 (H.M. Stationery Office), and "The Mechanical Production of Cold," by Ewing (Cambridge University Press).

FOODS PRESERVED IN TINNED IRON AND GLASS CONTAINERS.

A short account of the principles involved in the production of these materials is given in "Canned Foods in Relation to Health," by Savage (Camb. Univ. Press).

In the case of meat pastes, the meat is first boiled in an open vessel. It is next heated in an autoclave at 114° to 115° C. (237° to 239° F.), and finally, after being placed in the container, heated at 99° C. (210° F.). This final heating is known as *processing*, and the extent to which it is carried is determined by the nature of the contents of the container. Prolonged heating at a high temperature may lead to disintegration of the material. It should be noted that processing does not usually bring about sterilisation, that is, the *complete* destruction of *all* organisms.

In the present state of our knowledge as to the chemical changes which take place when putrefaction commences, the examination of preserved foods from a bacteriological point

of view is of more importance than a chemical examination. This question is dealt with from the former standpoint in another part of the course.* It may be mentioned, however, that food poisoning due to the consumption of "tinned" foods is due either to (*a*) the presence of bacteria or spores which have not been destroyed at the temperature employed in processing, and which become active owing, for example, to the admission of air to the container; or (*b*) as in the case of botulism, to poisonous substances produced by bacterial agency after supposed sterilisation, although the bacteria which have produced them may no longer be present. Very little is known as to the chemical nature of these very poisonous substances giving rise to botulism. There is reason for believing that they are changed on heating even to so low a temperature as 175° F., with the production of harmless substances; so that heating tinned food to the temperature of boiling water greatly minimises the risk of poisoning by the toxin of botulism, but such treatment does not destroy the spores. Substances of comparatively simple composition, known as ptomaines, such as putrescine (tetramethylene diamine, $NH_2(CH_2)_4NH_2$), and cadaverine (pentamethylene diamine, $NH_2(CH_2)_5NH_2$) have been isolated from the products of the decay of proteins, such as albumins, but the term ptomaine poisoning now appears to be used more or less in a popular sense to denote food poisoning in general.

The following extract from "Nature," of March 24th, 1923, with reference to botulism is of interest: "*B. botulinus* is poisonous only if it has been able to grow for some time under favourable conditions outside the body and produce large quantities of its potent toxin; man is poisoned by the toxin, not infected by the bacillus. Laboratory experiments show that the resting spores are exceptionally difficult to kill by heating. Considering, indeed, the wide distribution of the bacillus in nature, the rarity of botulism is a remarkable testimony to the care with which potted meats and so on are usually prepared. Really efficient sterilisation is a secure preventive."

Reference should also be made to the "Report on the Circumstances attending the Deaths of Eight Persons from Botulism at Loch Maree," by Leighton (H.M. Stationery Office, 1923), and the Annual Report of the Chief Medical

* See "The Bacteriology of Food," by C. Dukes (Lewis).

Officer of the Ministry of Health for 1922 (H.M. Stationery Office).

With reference to the use of tinned iron or glass for containers for food, although the latter is often preferred, it is possible that if the former has been employed a higher temperature has been reached in the final heating of the material in the container. In the case of glass containers, this final heating may have been curtailed, as it sometimes causes a separation of fat which detracts from the appearance of the material. In the case of tinned foods this is not obvious until the tin is opened.

From a chemical standpoint we shall deal here only with the examination of tinned foods as regards the question of metallic contamination.

Inspection of Tinned Foods.

With tinned foods it is often possible to condemn some samples by inspection of the container before the tin is opened, and sometimes a considerable proportion of a consignment is condemned at the port of entry.

If the container has been properly sealed and fermentation has not taken place, the pressure inside will be considerably less than atmospheric pressure, as the tins are usually sealed while hot. That is, the steam has displaced practically all the air from the container. If decomposition has set in, gases are produced, the pressure inside the tin rises, and the ends become convex, giving rise to what is known as a "blown" tin.

Inspectors of tinned foods are able to form an opinion as to the condition of the contents of a tin of meat by the character of the sound produced by tapping the surface of the tin. If there is a pressure inside the tin equal to, or greater than, that of the atmosphere, the contents will not be in contact with the whole of the container, and when this is the case a note of higher pitch is obtained at the points where the material does not touch the sides of the tin.

According to Savage and his co-workers,* the maintenance

of a partial vacuum in the container is of very great importance. They state: "*We have to regard canned meat and fish products as, at the best, only partly sterilised, and for the most part as containing viable bacilli, many of which are of a decomposing type. The food is sound rather on account of its being free from oxygen than because it is sterile.*"

It sometimes happens that air enters a tin owing to perforation due to rusting. This might, of course, lead to the introduction of organisms, but is probably of equal or even greater importance in supplying oxygen to bacteria already in the container which have not been destroyed in the process employed when the food was put in the container.

It is, of course, well known that these metal containers are made of thin sheet *iron* covered with *tin*, the latter simply acting as a protective layer inside and outside for the iron, the latter being a cheaper and harder metal. If, however, the tin is removed from the surface of the iron over a small area, as may easily happen with tin plate of poor quality, corrosion of the *iron* at this point takes place more rapidly than if the tin were not in contact with the iron, and a small hole through the plate may be made in this way. It should be noted, on the other hand, that with galvanised iron (iron coated with zinc) the zinc is more easily attacked than the iron. Zinc-coated vessels are, however, unsuitable for use as food containers, as zinc is more easily attacked by acids and alkalis than tin.

The Action of Tinned Food on the Container.

Although tin is present in most canned foods, owing to the action of acids on the container, the amount is usually very small, even after the food has been kept in tins for several years. This amount is usually less than 2 grains per pound (i.e. 2 parts per 7000), and if not present in excess of this amount, is probably harmless, unless a very large portion of such a food is consumed at one time. Tin is, however, a poisonous metal, and cases are recorded where from 2 to 5 grains of tin taken at one time in tinned foods have produced acute irritant poisoning. If salts of the metal are taken regularly in very small doses over a considerable period, most of the tin appears to be excreted (see Report to Local Government Board "On the Presence of Tin in Canned Foods," by Buchanan and Schryver (H.M. Stationery Office, 1908).

Tin is not readily attacked by solutions having low hydrogen ion concentration (such as solutions of most organic acids), and it is certain that the presence of colloidal matter affords protection to the metal (see reference to protection of aluminium, p. 215).

To illustrate this point take two pieces of thin tin foil (not tinned iron) about 4 inches long and 1 inch wide. Roll these round a glass tube lengthways into cylindrical form, so as to fit loosely into test tubes.

Fill one test tube (A) with N/50 hydrochloric acid diluted with an equal volume of distilled water, and the other (B) with N/50 hydrochloric acid diluted with an equal volume of 0·5 per cent. gelatine solution. Heat the test tubes containing the tin foil and solutions in a boiling water bath for about one hour.

Pour off the solutions, after cooling, into Nessler cylinders, and dilute the acid solution from the tube (A), which did not contain gelatine, with an equal volume of 0·25 per cent. gelatine solution, and the other solution from (B) with an equal volume of distilled water. Both solutions now contain the same amount of gelatine. Add sulphuretted hydrogen solution to both Nessler cylinders, when it will probably be found that tin sulphide is produced only in the solution which did not contain gelatine during its action on the tin.

In order that the results shall be strictly comparable, it would be necessary to ensure that the hydrogen ion concentration in both solutions is the same when in contact with the tin. The reason for adding gelatine to solution (A) before the addition of hydrogen sulphide is that metallic sulphides are sometimes not completely precipitated in the presence of colloids.

Soldering of tins.—Although the tin usually gets into the food from the container, occasionally its presence is due to the entrance of solder, a mixture of tin and lead, into the container. The tins in which food is to be preserved are usually made in such a way that the solder along the folded seams is on the outside, and a " solder trap " is often placed under the vent hole to prevent liquid solder dropping on to the contents of the container when this is finally sealed. If solder enters, the amount of tin dissolved is greatly increased, and, in addition, there is the possibility of solution of the more poisonous

metal lead. Probably at first the presence of the lead only accelerates the solution of the tin.

It should be noted that it has been found that when tin is dissolved from a container, compounds of the metal accumulate in the *solid* particles of the material.

POISONOUS METALS IN FOODS.

Tests for the detection of tin, lead, copper, and arsenic will be described. It should be noted, however, that these metals are by no means the only ones of which the compounds are poisonous. For example, salts of barium and mercury are poisonous, but under ordinary circumstances foods are unlikely to be contaminated by these substances.

In view of the extensive use of aluminium for cooking vessels, the corrosion of this metal is also dealt with briefly, although there is no reason to suppose that the minute quantity of the metal with which food may become contaminated in ordinary culinary operations has any significance.

Detection and Determination of Tin in Foods.

The organic matter must first be destroyed. This may be effected as in the Kjeldahl process (p. 11), by heating with concentrated sulphuric acid and potassium sulphate in a large flask, 20 to 50 gms. of the food material being employed. It is an advantage to use diluted sulphuric acid at first to hydrolyse the proteins, the final decomposition being effected by concentration of the acid and the addition of more acid to the cooled flask as required.

After dilution the tin is then precipitated as sulphide, and weighed as oxide (see Vol. I., p. 105). If other metals, such as lead and copper, are present which are precipitated by hydrogen sulphide, the separation of tin sulphide is effected by solution in sodium hydroxide and reprecipitation by means of acid.

A colorimetric method for the determination of tin is described in the Report by Buchanan and Schryver, referred to on page 208.

Lead in Foods.

It is well known that lead is a cumulative poison, and therefore it is of the greatest importance that food should be free from even traces of lead compounds.

The possibility of the introduction of lead from solder into a tinned food has been referred to.

It is shown in Volume I. that contamination of food by lead may take place when a food is cooked in certain casseroles. It is probable that the presence of colloidal matter in this case also diminishes the solvent action of the acid liquid on the lead compounds of the glaze, so that the amount of lead taken up by the food cooked in these vessels is less than would be expected from the acidity of the liquids. Some results which have been obtained show, however, that some lead is extracted when acid foods such as tomatoes are cooked in these vessels (see H. Masters, "The Lancet," 1920, I., 1394; also Public Health Report, No. 29, 1925, "The Solubility of Glazes and Enamels in Cooking Utensils," by Monier Williams, H.M. Stationery Office, 6d.

Tartaric acid, cream of tartar, etc., often contain traces of lead taken up in their manufacture, but the maximum amount allowed in such materials is *one-seventh of a grain* per pound.

Another method by which lead compounds may be introduced is dealt with under colouring matters (p. 148). A sample of egg powder (a coloured baking powder) examined in this laboratory was found to contain 0·5 per cent. of lead chromate (chrome yellow) as colouring matter. It was found, on investigation, that this particular kind of egg powder had been made and sold on a small scale for over twenty years. Fortunately, such a case is probably quite exceptional, but under present conditions in this country it is possible that such a thing might happen again. Chrome yellow can be purchased from a paint shop, and is not labelled poison.

If, however, the Draft Rules (p. 203) are adopted, the use of such a substance as colouring matter would be illegal.

Determination of Lead in Foods.

The amount of lead in a foodstuff is determined colorimetrically as sulphide (see Vol. I., p. 30), but it is first necessary to destroy the organic matter. This can be effected by charring the material in a porcelain basin, adding concentrated sulphuric acid when cold, and again heating. A little concentrated nitric acid may be added to facilitate the removal of the carbon, but the dish must be allowed to cool before any acid is added. On evaporation to dryness no particles of carbon should be visible in the ash.

If lead was present in the original substance, it is now in the form of lead sulphate, which may be extracted from the ash by means of a solution of ammonium acetate containing ammonia. If copper was present, this metal may also be in the extract obtained on extraction with ammonium acetate, but the addition of potassium cyanide prevents precipitation of copper sulphide when the lead sulphide is precipitated by the addition of sulphuretted hydrogen (see Vol. I., p. 31).

As the amount of lead present will probably be exceedingly small, at least 100 gms. of the food should be taken for the test, and the removal of the organic matter will require a considerable time.

Copper in Foods.

Compounds of this metal are present in minute traces in several foods, but the chief point of interest in connection with copper in foods is the use of salts of the metal for imparting a green colour to preserved peas, etc.

Copper salts combine with proteins in peas, French beans, etc., with the production of compounds, with intense green colours, and it should be noted that the colour of materials which have been treated with copper salts is not due to the chlorophyll originally present.

The only possible justification for the addition of traces of a poisonous metal such as copper to a food is that by its addition the food is given a more appetising appearance, and the possibility that the copper compound formed may pass through the body unchanged; the latter supposition has, however, not been definitely proved.

The Departmental Committee appointed in 1899 by the Local Government Board to enquire into the use of preservatives and colouring matters in connection with foods reported against the employment of copper compounds for such purposes. At the present time the practice appears to be allowed, if the amount does not exceed 0·5 grain of copper per pound, and its presence is notified on the label. It is probable, however, that such addition of any copper compound will shortly be illegal.

Another possible source of contamination of food by copper is the use of a copper preserving pan which has become corroded. Compounds of copper such as basic carbonate, which are produced under certain conditions on copper vessels, are

much more soluble in organic acids than is the metal itself. It is very important, therefore, that a copper vessel used for cooking food should be quite bright and free from corrosion. The solubility in dilute acids of copper compounds, which are present on the surface of the tarnished metal, may be shown by means of simple comparative experiments with pieces of bright and tarnished copper foil. If these are allowed to remain in dilute acid solutions for a short time, the presence of copper in solution, in the case of the tarnished foil, may be shown by the formation of a brown coloration, due to copper sulphide, on the addition of sulphuretted hydrogen.

If copper is to be preserved from corrosion it should not be covered with fat, as fatty acids liberated by the hydrolysis of the fat attack the metal. A thin protective layer of vaseline (a mixture of hydrocarbons) is suitable for this purpose.

Detection of Copper in Dried Peas.

About 10 gms. of the material are heated in a porcelain or, preferably, platinum basin until thoroughly charred. When cold, the charred mass is extracted with about 20 c.c. of dilute hydrochloric acid and filtered. (The extraction in this way of part of the mineral matter from the charred material shortens the time required for the removal of all the carbon in the subsequent heating.) The filter paper and residue are then dried and burnt to an ash. The hydrochloric acid extract is added to the cold ash, and the mixture heated for about a quarter of an hour on a boiling water bath. A small quantity of water is then added, the solution filtered, and the filtrate evaporated to dryness on the water bath. The residue obtained is dissolved in a small quantity of water containing two drops of dilute hydrochloric acid, and tested for copper by the addition of ammonia and sulphuretted hydrogen solutions. A brown coloration, removed on the addition of potassium cyanide solution, indicates the presence of copper. If the coloration is due to iron it is removed on addition of hydrochloric acid. The liquid containing the sulphide in suspension is therefore divided into two parts, one portion being treated with hydrochloric acid and the other with potassium cyanide solution. The presence of copper may also be indicated by the appearance of a blue coloration on the addition of the ammonia before the sulphuretted hydrogen is added.

In the absence of lead, the copper may be determined

colorimetrically as sulphide, by matching the colour produced with that produced by a known amount of a standard solution of copper sulphate. It may also be determined by electrolysis of the solution of the ash containing nitric acid. In this process the electrolysis is carried out in a weighed platinum dish, which acts as the cathode, a coil of platinum wire being used as anode. A current of about 0·3 amperes should be used, and should pass through the solution for several hours. The dish is then washed and dried in the steam oven, and the increase in weight gives the weight of copper in the material taken for the test.

Zinc in Foods.

Minute traces of this metal are present naturally in certain foods, but the metal is more likely to be introduced by storage of food in galvanised iron vessels, or as zinc chloride ("killed spirits of salts," i.e. hydrochloric acid which has been treated with zinc until no further action takes place), which is sometimes used as a flux in soldering.

The metal is tested for in the solution of the ash, obtained as described under the test for the presence of copper in peas (p. 213), by the ordinary methods of qualitative analysis. That is its sulphide is precipitated in Group III.B after removal of metals in Groups I., II., and III.A.

Aluminium in Foods.

This metal is undoubtedly less toxic than the other metals previously dealt with. There is apparently no evidence that the minute quantity of the metal such as will be dissolved from aluminium cooking vessels is of any significance, although taken in relatively large quantities it would probably be harmful.

An investigation has been undertaken by the authors with reference to the black "stain" often produced on an aluminium saucepan when tap water is boiled in it. This appears to be due to the removal of a *very* thin film of aluminium owing to the alkalinity of the water, with formation of an aluminate (see Vol. I., p. 274). The impurities in the metal (iron, etc.) remain undissolved and produce the stained appearance. Distilled water does not produce the black stain, as it is practically without action on the metal.

The amount of aluminium removed is very small indeed,

even when extensive blackening occurs. It cannot, however, be assumed that the extent of the blackening affords a measure of the amount of aluminium removed, as by the action of a dilute solution of sodium hydroxide on the metal, which is much greater than that of tap water, the black deposit is removed, remaining in suspension in the liquid, and the metal is free from stain.

The attack on the aluminium is much less pronounced in presence of colloidal material. Thus an aluminium saucepan which shows extensive discoloration when tap-water is boiled in it, is often unaffected when colloidal substances, such as would be met with in cooking, are present in the water. This may be illustrated by boiling (*a*) potatoes in their skins, (*b*) peeled potatoes in tap water in an aluminium vessel. Much less blackening of the vessel will be observed in (*a*) owing to the extraction of colloidal matter from the patato.

The protection of aluminium from the action of alkali in the presence of sodium silicate is probably to be explained either by the presence of colloidal matter, or by the formation of a protective layer of aluminium silicate on the surface of the metal. This is of importance in connection with the cleaning of aluminium vessels with "soda." See Seligman and Williams, "J. Inst. Metals," 1922, 297; "Analyst," 1922, **47**, 493.

If potassium dichromate solution is boiled for some time in an aluminium saucepan the metal becomes "passive," and remains so for some time; so that if tap-water is boiled in a saucepan which has been treated in this way, no black stain is apparent.

It is well known that the black stain on an aluminium saucepan is removed if an acid liquid such as a fruit juice is heated in the saucepan, the explanation being that the iron is dissolved in the acid (see paper by Tinkler and Masters, "The Analyst," 1924, **49**, 30).

Another source of contamination of food by aluminium compounds is referred to in connection with bread-making. The use of alum in this connection, however, has now probably been abandoned almost entirely (see p. 124).

Arsenic in Foods.

It is a matter of common knowledge that compounds of arsenic are extremely poisonous, but fortunately the detection and determination of arsenic in chemical substances

likely to be used in connection with the preparation of foods and beverages is a matter of comparative simplicity. The importance of this examination is now *usually* recognised by those making use of these materials in preparation of food products.

The most frequent source of contamination of food by arsenic is in connection with the use of sulphuric acid in the manufacture of glucose (see p. 111), some of the arsenic in the iron pyrites, etc., from which the acid is made eventually finding its way into a food or beverage.

A large number of cases of arsenical poisoning occurred in 1900, due to drinking beer in connection with the manufacture of which glucose, containing a considerable quantity of arsenic, had been used.

Sulphuric acid, or hydrochloric acid made by means of it, is also used in connection with the manufacture of acid phosphates for use in baking powders (see p. 131), boric acid for preservatives, organic colouring matters, etc.; so that arsenic may be present in any of these materials. Recently the presence of traces of arsenic in a particular consignment of cocoa was found to be due to the employment, in one stage of its manufacture, of potassium carbonate, which was contaminated with a compound of arsenic (see p. 195).

It will thus be apparent that it is absolutely essential that any chemical substance used in connection with the manufacture of a food or beverage should be tested for the presence of arsenic.

As a rule, the amount of arsenic in any food or beverage is extremely minute, and does not exceed *one-hundredth of a grain* (expressed as arsenious oxide, As_4O_6) per pound of a solid (1 part in 700,000), and one-hundredth of a grain per gallon of a beverage (1 part in 7,000,000). These are the maximum limits recommended by the Royal Commission on Arsenical Poisoning (1903). A fatal dose of arsenious oxide taken at one time is probably from 2 to 3 grains (0·13 to 0·2 gm.).

The principle underlying the two chief methods for the detection of arsenic is the same. When compounds of this element are introduced into a solution in which hydrogen is being generated, either by the action of an acid on zinc, or electrolytically, the arsenic is converted into gaseous arseniuretted hydrogen, AsH_3. In the case of a foodstuff, etc., a preliminary treatment is usually necessary, which is often a matter

of considerable difficulty. Thus it is necessary to destroy organic matter by oxidation, and to oxidise sulphur compounds to sulphuric acid, thereby removing the possibility of evolution of hydrogen sulphide. This oxidation can be effected by means of hydrochloric acid and potassium chlorate, or nitric and sulphuric acids.

In the *Gutzeit* test, the arseniuretted hydrogen is allowed to act on mercuric chloride, forming an orange coloured substance. The coloration obtained can be compared with that obtained by employing a known amount of an arsenic compound. This method is described in detail below.

In the *Marsh-Berzelius* test, the gas is decomposed by passing through a heated hard glass tube, a mirror of arsenic being obtained on the cool part of the tube. This is the method employed in a toxicological examination; as little as 0·01 mg. ($\frac{1}{6,500}$ grain) of arsenious oxide giving a very distinct mirror. The amount of arsenic is determined by comparing the mirror obtained in the test with those produced from known amounts of arsenious oxide.

Although the principles involved in the detection and determination of arsenic by this method are so simple, a great deal of experience of the method is necessary if reliable results are to be obtained.

For further information on this subject, see Report of Royal Commission on Arsenical Poisoning (H.M. Stationery Office), 1903; "British Pharmacopœia," 1914; and Report of Joint Meeting of the Society of Public Analysts and the Nottingham Section of the Society of Chemical Industry; "The Analyst," 1923, **48**, 63.

The Gutzeit Test for Arsenic.

If arseniuretted hydrogen is brought into contact with mercuric chloride a yellow coloration is produced, owing to the formation of a compound represented by the formula $As(HgCl)_3$ as follows:—

$$AsH_3 + 3HgCl_2 \rightarrow As(HgCl)_3 + 3HCl.$$

In carrying out the test, a bottle of approximately 120 c.c. capacity is fitted with a rubber bung carrying a glass tube about 20 cm. long and 5 mm. internal diameter. The upper end of the tube is widened to about 8 mm. diameter, and the lower end is drawn out to about 1 mm. internal diameter.

A hole about 2 mm. diameter is blown in the side of the tube near the lower end. This arrangement will prevent a spray of acid entering the tube.

A piece of filter paper about 10 × 4 cm. which has been soaked in a solution of lead acetate and dried, is rolled into a small coil (4 cm. long), and placed in the glass tube, so that its upper end is about 2 cm. below the top of the tube. Any sulphuretted hydrogen liberated in the bottle will be absorbed in this way, with formation of lead sulphide.

A disc of filter paper 5 cm. in diameter, soaked in a solution of mercuric chloride and then dried, is fastened over the end of the tube by folding down the edges of the disc and securing with a rubber band, cut from a piece of rubber tubing. (These papers should be kept in a bottle in the dark.)

A standard solution of arsenious oxide in hydrochloric acid is required in carrying out the test quantitatively. For this purpose 1 gm. of pure arsenious oxide is added to about 50 c.c. of water containing 1·5 c.c. of concentrated hydrochloric acid.* The mixture is warmed until a clear solution is obtained, when it is cooled and diluted to 100 c.c. 1 c.c. of this solution = 0·01 gm. of arsenious oxide.

1 c.c. of this solution diluted with water to 1000 c.c. gives a solution of which 1 c.c. is equivalent to 0·00001 gm. (0·01 mg.) of arsenious oxide.

This very dilute solution is the one used in this test and in the Marsh-Berzelius test for comparative purposes.

To ascertain if the reagents and apparatus are free from arsenic, 50 c.c. of hot water are placed in the bottle of the apparatus, and 10 c.c. of hydrochloric acid, As.T. (specific gravity, 1·16), 1 drop of stannous chloride solution, As.T., and 10 gms. of zinc As.T. added.† The rubber bung, carrying the tube with lead acetate and mercuric chloride papers, is then replaced, and the action allowed to proceed for half an hour. The mercuric chloride paper should be protected from direct sunlight during the test.

If no stain is produced on the mercuric chloride paper the materials are free from arsenic. If an appreciable stain is produced other reagents must be employed.

* It is obvious that the reagents employed must be free from arsenic or contain this element in extremely minute quantity. Such reagents can now be obtained, and are labelled "As.T." (arsenic test).

† The addition of stannous chloride accelerates the evolution of hydrogen, a small amount of tin being liberated on the surface of the zinc (compare Vol. I., p. 11).

The test is then repeated, using 1 c.c. of the dilute solution of arsenious oxide in addition to the materials previously employed.

The stain on the mercuric chloride paper now obtained corresponds to 0·01 mg. of arsenious oxide, and will be referred to as the standard stain.

Examination of Glucose for the presence of Arsenic.

50 c.c. of hot water are added to approximately 5 gms. (weighed to the nearest 0·01 gm.) of the syrup, or solid glucose contained in the bottle of the Gutzeit test apparatus. The syrup is weighed in a small beaker containing a rod (see p. 112), the amount taken being obtained from the loss in weight.

When the glucose has dissolved, 0·5 c.c. of bromine solution, As.T., and 10 c.c. of hydrochloric acid, As.T., are added. The mixture is allowed to stand for five minutes, which will lead to the oxidation by the bromine of any sulphite used as preservative, the bromine reacting with the water, to supplying the necessary oxygen :—

$$Br_2 + H_2O \rightarrow 2HBr + O$$
$$NaHSO_3 + O \rightarrow NaHSO_4.$$

If the sulphite were not removed, a relatively large amount of sulphuretted hydrogen would be liberated when the zinc is added. If this gas came in contact with the mercuric chloride paper a stain, due to the formation of mercuric sulphide, would be produced, which is not unlike the stain due to the presence of arseniuretted hydrogen.

A few drops of stannous chloride solution, As.T., are next added to remove the excess of bromine—

$$SnCl_2 + Br_2 \rightarrow SnCl_2Br_2.$$

10 gms. of zinc As.T. are then added, and the rubber bung carrying the glass tube with lead acetate and mercuric chloride papers is fitted to the bottle.

The action is allowed to proceed for thirty to forty minutes, in a place in which it is not exposed to direct sunlight.

On the expiration of this time the stain on the mercuric chloride paper is compared with the standard stain obtained above.

[To make certain that the stain on the mercuric chloride paper is due to arsenic, the paper on which the stain has been

produced is boiled with concentrated hydrochloric acid. By this treatment, if the stain is due to arsenic, it turns a brick-red colour, grey if due to antimony, and disappears if due to mercuric sulphide.]

If the stain is deeper than that of the standard obtained from 1 c.c. of the dilute solution of arsenious oxide (1 c.c. = 0·01 mg. As_4O_6), then the 5 gms. of material taken for the test must contain more than 0·01 mg. of arsenious oxide,

i.e. 10 gms. contain more than 0·00002 gm. As_4O_6,
or 1,000,000 ,, ,, 2 gms. As_4O_6.

The British Pharmacopœia limit for arsenic in glucose is 2 parts per million. One hundredth of a grain per pound (0·01 grain in 7,000 grains), the limit recommended by the Royal Commission on Arsenical Poisoning corresponds to 2 parts in 1,400,000.

Chapter IX.

* * THE COOKING OF FOODS.

In dealing with this subject it is as well to realise that the cooking of foods is still primarily an art and not a science, and that our knowledge of the chemical and physical changes which take place during the various processes employed in the preparation and cooking of food is at present extremely meagre.

This is hardly surprising when the highly complex nature of the materials which have to be dealt with are taken into consideration, since not only their chemical composition, but also their biological structure must be taken into account.

A scientific study of this subject is further rendered more difficult by the fact that there is no one general underlying principle involved in the cooking of foods, since some foods are cooked for one reason and some for another, and the methods of cooking used are modified accordingly.

Thus vegetable foods, which consist largely of cellulose and starch, are cooked with a view to rendering them more digestible, and the main object of the cooking process is to bring about the softening and mechanical breaking down of the cellulose, and the swelling and gelatinisation of the starch grains. For this reason such foods are nearly always cooked by heating them in contact with boiling water or steam.

The digestibility of meat, on the other hand, is not increased by cooking, since the proteins are coagulated on heating, and thus become less easily soluble. By suitable methods of cooking, however, the flavour in the meat can be developed, and to the average taste it becomes much more palatable; but this will only be the case if the method of cooking is such that the meat bases or extractives, on which the flavour largely depend, are retained in the meat. Hence meat is most frequently cooked by roasting or grilling, and when it is cooked in contact with water it is usual to serve the cooking liquor

with the meat, so that the loss of extractives may be reduced as far as possible.

In some cases, e.g. milk, cooking may be regarded merely as a method of preserving the food by destroying organisms; or the food may be cooked simply because it is considered to be more appetising when served warm. For this purpose it is only necessary to supply heat in such a manner that the temperature of the food may be raised without producing undue decomposition.

To give some idea of the numerous difficulties which arise in attempting any practical study of the changes which take place in food on cooking, one factor only, namely, change of weight, may be taken in illustration.

On roasting meat there is a loss in weight, due chiefly to the evaporation of water and the oozing out of other constituents. Vegetables, on the other hand, when cooked in water, lose a proportion of their soluble constituents, but take up water, and often show a gain in weight on cooking.

In both cases this change in weight will be influenced, not only by the variety and condition of the meat or vegetable used, but also by the method, temperature, and rate of cooking.

The means by which the heat is supplied should also be taken into account, since in a coal- or in an electrically-heated oven, the heating is chiefly by radiation, whereas in the ordinary type of gas cooker the heating is chiefly by convection, and it is unlikely that uniform results would be obtained under such different conditions.

When the foods are cooked in contact with water, the natural properties of the water, e.g. the presence or absence of certain mineral salts, may introduce yet another variant.

Further, although it is obvious that the change in weight must be largely dependent on the time of cooking, it is impossible to lay down any standard for determining exactly when a food is *cooked*, and this, in most cases, is largely a matter for individual judgment.

If the temperature conditions are kept constant, it may be possible to regulate the cooking to some extent by timing. It is difficult, however, to draw up any practical scheme which will make due allowance not only for the weight, but also for the external surface of the food material.

In roasting a large joint of meat, for example, the external surface is, in general, relatively smaller than in the case of a small joint, and the loss by evaporation, etc., is likely to be

relatively less in the former than in the latter case. On this account, also, it is usual to regulate the temperature rather differently in the two cases.

The time required for cooking also depends on the *condition* of food, e.g. young green peas cook much more rapidly than old peas, and similar differences which, in many cases, it may not be possible to detect beforehand, are likely to occur in other foods.

Reliable data can, in fact, only be obtained by taking the average result of a large number of experiments, which have been carried out under carefully controlled and exactly similar conditions.

During recent years considerable progress has been made in the collection of such data, and the results thus obtained will be discussed during the course of the lectures, but it is necessary to point out that although this type of investigation presents an interesting field to those who can undertake it as research work, the subject is one in which it is by no means easy to devise instructive experiments which can be carried out in the time and with the equipment available for class work in the laboratory.

The practical work described in this section has therefore been confined to a selection of experiments which experience has shown can be carried out either by a class working in the laboratory, or, if preferred, as demonstrations. These experiments are also designed to give instruction with regard to data already established in connection with the cooking of foods and the methods which have been employed in carrying out such investigations.

A number of publications are referred to in the text, but mention should also be made of the Bulletins, relating to the preparation and cooking of different foods, which are published from time to time by the Department of Agriculture of the United States of America, in which the results obtained at various experimental stations are recorded.

The characteristic changes which occur on heating proteins and carbohydrates have already been mentioned in the foregoing chapters, and it is hardly necessary here to describe experiments to show that proteins coagulate on heating, and that starch grains swell and undergo hydrolytic changes on heating with water. In view, however, of the conflicting statements which are sometimes made with regard to changes

which take place in fats on cooking, the behaviour of fats on heating may be briefly considered here. Fat which is free from water can be heated to a high temperature without undergoing any marked decomposition, and fat which is used for frying is usually heated to a temperature of about 250° C. At this temperature a faint blue vapour rises from the surface, showing that incipient decomposition is taking place, and that some of the more volatile decomposition products are being liberated. If the temperature is raised much above this the fat is liable, if heated over a flame, to ignite and burn, and so disintegrate completely.

Statements have from time to time appeared in various books dealing with dietetics to the effect that fats, when cooked in contact with water, undergo hydrolytic changes.

These views were not confirmed by some investigations, carried out in this department, on the nature of the changes which occur when fats are cooked in contact with flour and water, in the form of pastry.*

In these experiments both butter fat and cotton seed oil were used, and the constants, namely, saponification value, iodine value, acetyl value, and refractive index, of the fat and oil were determined before and after cooking.

There was no marked change either in the saponification value or in the acidity after cooking, which should be the case if hydrolysis is taking place, and the changes in the other constants were also slight. The most definite change observed was the production of small amounts of "oxidised" acids, such as are produced in blown oils, i.e. oils which have been oxidised by blowing air through them.

As might be expected, the cotton seed oil gave larger amounts of these acids than the more saturated butter fat.

In recent years we have learnt that the value of a food does not depend entirely on the amount and character of the food constituents present, but that food can only act efficiently as such if certain other substances known as *vitamins* or *accessory food factors* form part of the diet. The exact nature of these substances has yet to be determined, and their presence or absence in different kinds of foods can at present only be detected by means of feeding experiments. The question of the extent to which these bodies are likely to be

* Smith and Masters, "The Analyst," 1914, 39, 347.

affected by the processes ordinarily used in cooking raises yet another field of enquiry in connection with the cooking of food. Since, however, chemical methods of investigating such changes have yet to be devised, the subject is one which must at present be left chiefly to the physiologist and dietician, and is not one which can be suitably dealt with in the chemical laboratory. It would appear that the stability of these substances towards heat is influenced by a number of different factors, which include degree of temperature, time of heating, presence or absence of oyxgen, and in some cases of acids and alkalis; but that, as a general rule, a proportion at least of the vitamin is likely to escape destruction during the process of cooking. Further, if foods are taken in normal quantities, and prolonged cooking is avoided, it is probable that enough vitamin will be left even after cooking to meet the ordinary needs of the diet.

For further information, reference should be made to "The Vitamine Manual," Eddy (Williams & Wilkins); and to "Report on Present State of Knowledge of Accessory Factors (Vitamins)," second edition (H.M. Stationery Office,) 1924.

SUGAR BOILING AND CONFECTIONERY PROCESSES.

Cane sugar may be regarded as exceptional, in that it is a food composed of an individual compound of known chemical composition, which is usually obtained in an almost chemically pure condition (see p. 108).

Some of the changes which take place when sugar is heated can be demonstrated in the laboratory, and experiments may be carried out to illustrate the methods by which these changes are regulated and utilised in the preparation of various confectionery products.

Pure cane sugar is soluble in about half its own weight of water at room temperature, and its solubility increases with rise of temperature. On evaporation the sugar crystallises out in colourless transparent crystals.

When a solution of sugar in water is heated the physical properties of the mixture alter as the temperature rises, and definite *stages* or *degrees* of heating based on these changes in properties are recognised by the sugar boiler. A sugar boiling thermometer graduated in degrees Fahrenheit is now generally used to regulate the heating of the sugar solution. The terms

used by the sugar boiler, however, refer to practical tests, by means of which the experienced worker can judge, without a thermometer, to what *degree* or *stage* the sugar has been heated.

On a large scale sugar boiling is usually carried out in large copper pans which are heated either over a fire or over gas, and the sugar is mixed with water in the proportion of 7 lb. of sugar to 1 quart of water.

Working on the small scale in the laboratory, 250 gms. of white granulated sugar may be heated with 100 c.c. of water in an enamelled saucepan. The mixture should be gently stirred until all the sugar has dissolved.

At a temperature slightly above 212° F., the boiling-point of water, the solution begins to boil, but on continuing the heating the temperature steadily rises, and samples of the liquid may be withdrawn at intervals from the pan and tested. As the temperature rises the solution of sugar will, of course, become more concentrated, owing to the evaporation of water, and the physical properties of the mixture change.

The tests employed at the different stages, and the temperatures with which these stages or degrees should approximately correspond, are given below :—

Stages or Degrees of Sugar Boiling.

Smooth Degree, 215°-220° F.—Some of the sugar is withdrawn on a clay pipe-stem or glass rod, and is gently drawn along the rod between the finger and thumb. The sugar feels oily or *smooth*, and hence the name of the degree.

Thread Degree, 230°-235° F.—The solution at this stage is sufficiently viscous that it can be drawn into a short *thread* if a little is pulled out between the finger and thumb.

Blow or Feather Degree, 240°-245° F.—The liquid becomes so viscous that the steam generated *blows* the mass into large bubbles, and the liquid shows a tendency to boil over.

If some of the liquid is removed with a small wire strainer, then on blowing gently through the perforations the sugar should separate in small *feathery* particles.

Ball or Pearl Degree, 250°-255° F.—Some of the sugar is removed on a glass rod and dipped into cold water. The sugar should then be of about the same consistency as putty, and can be moulded between the finger and thumb to form a small *ball* or *pearl*.

Crack Degree, 310°-316° F.—The sugar, if cooled by dropping into cold water, rapidly hardens, becomes brittle, and can be readily *cracked* or splintered. The sugar at this stage is no longer colourless, but is assuming a yellow colour, and if the heating is continued it rapidly darkens or begins to *caramelise*. At a temperature of about 360° to 380° F. it is largely converted to a dark coloured substance known as *caramel*.

During the latter part of the process of heating, the sugar has been gradually splitting off water, but it will have retained throughout the character of a carbohydrate. The formula usually assigned to *caramelan*, which is the characteristic constituent of caramel, is $C_{12}H_{18}O_9$, so that the conversion of cane sugar to caramelan on heating may be represented thus :—

$$\underset{\text{Cane Sugar.}}{C_{12}H_{22}O_{11}} \rightarrow \underset{\text{Caramelan.}}{C_{12}H_{18}O_9} + 2H_2O.$$

"Cutting the Grain."

Sugar which has been heated with water, in the manner described, to above 240° F., will, on cooling, crystallise to form a hard, granular mass. To "cut" or destroy this tendency to "grain," the confectioner usually adds to the sugar, before heating, a small quantity of some organic acid, e.g. tartaric acid, cream of tartar, or citric acid.

The sugar thus treated forms, on cooling, a vitreous non-crystalline mass, and does not readily crystallise on keeping.

On heating the sugar with the acid, some of the cane sugar will be converted into invert sugar (see p. 71), and as invert sugar crystallises only with difficulty, the presence of a proportion of invert sugar in the mixture has the effect of preventing, or at least considerably delaying, the crystallisation of the sugar.

To illustrate this point the following experiment may be made :—

To 30 gms. of cane sugar add 10 c.c. of water, and warm until a clear solution is obtained.

Divide the solution into two equal portions. To one portion add 1 c.c. of 10 per cent. tartaric acid solution, and warm on a water bath to convert the cane sugar to invert sugar. To the other portion add 1 c.c. of water, so that the two solutions will be of equal concentration, and warm for the same length of time.

Set both solutions aside and observe that after some time the cane sugar solution begins to crystallise, and if left long enough becomes almost solid; whereas the solution of invert sugar does not crystallise.

Invert Sugar in Jam and Honey.—When fruit is heated with sugar in the preparation of jam, there is usually sufficient acid in the fruit juice to ensure the formation of enough invert sugar to prevent the crystallisation of the jam on keeping. Jam does, however, sometimes become "sugary" owing to lack of acidity in the fruit.

Although solutions of invert sugar or mixtures of cane sugar and invert sugar do not crystallise readily, *concentrated* solutions of invert sugar may, on keeping for a considerable time, crystallise out.

This is seen in the case of honey, which is essentially a concentrated solution of invert sugar, since honey, after keeping some months, often crystallises to form a hard white mass.

Samples of artificial honey prepared in the laboratory by Herzfeld's method (see p. 110) have also been observed to crystallise after keeping for about twelve months.

Experiments may now be made to illustrate the processes used in the production of two different types of sweetmeat:—

(1) Barley sugar, or transparent boiled sugar goods.
(2) Fondants.

Barley Sugar.

Heat 100 gms. of white granulated sugar with 50 c.c. of water in a small enamelled saucepan, and stir until the sugar has completely dissolved.

If a sugar-boiling thermometer is not available, an ordinary thermometer graduated either in degrees Fahrenheit or in degrees centigrade may be used, but in the latter case all the given temperatures must be converted to the centigrade scale.

The bulb of the thermometer may be protected by encasing the thermometer in a glass tube, sealed at the lower end, so that, if the thermometer is used for stirring, the bulb does not come into direct contact with the bottom of the pan. When the temperature reaches 240° F. "the grain" should be "cut," but before this is done a small portion of the sugar may be withdrawn. This should afterwards be heated separately without

the addition of acid, i.e. without "cutting the grain," and its behaviour on keeping compared with that of the remainder of the sugar in which the grain has been cut.

Thus, when the temperature of the liquid reaches 240° F., transfer a small portion to a beaker, and to the remainder add 0·2 gm. of tartaric acid, and heat to the *crack degree*, 310° F. In carrying out experiments in the laboratory, the lower limits of the temperatures given for the different degrees should be adhered to, as owing to relatively greater loss of water by evaporation when working on a small scale, the sugar will probably reach the required consistency at a rather lower temperature than is normally the case.

Pour out the syrup slowly on to a greased slab or into a flat-bottomed dish. Allow the sugar to cool, and whilst it is still fairly plastic, cut into strips with a sharp knife. Raise the strips carefully off the slab with the knife, and to produce the characteristic spiral appearance usually associated with barley sugar, twist the strips between the finger and thumb.

The sample of sugar which was removed before cutting the grain should now be heated to 310° F., and treated in the same way as before. It will be observed that on keeping for a few days this material loses its transparent appearance and becomes "sugary."

Acid drops, *fruit drops*, *etc.*, are also prepared by the same process, different colouring and flavouring matters are added and the sugar is poured out to form drops, or if a special shape is desired, poured into moulds.

Toffee is a preparation of a similar character, and differs only in containing butter or other fat as an additional ingredient.

Fondants.

Heat 250 gms. of sugar with 100 c.c. of water and 0·3 gm. of cream of tartar to a temperature of 240° F. (Feather degree).

Pour the syrup out into a glazed earthenware basin and allow it to cool to about 100° F.

Remove a small portion of the syrup and set it aside for comparison. Stir the remainder of the syrup with a wooden spoon; and keep working the sugar from the sides of the basin towards the centre. The sugar becomes more and more viscous, and also less transparent and more creamy in appearance.

When the sugar becomes too stiff to be stirred with the

spoon, knead it with the hands until a smooth creamy mass of about the same consistency as a soft putty is obtained. In this condition the sugar is known as *fondant*, and is used as the basis of a number of different sweetmeats, and also for other confectionery processes, e.g. coating cakes.

The observed changes in the texture of the sugar are to be attributed to the separation of minute crystals of cane sugar, and the fondant may be regarded as a suspension of fine cane sugar crystals in a syrup of non-crystallised sugar.

This partial crystallisation of the sugar only takes place if the sugar is stirred, and it will be found that the sample which was set aside for comparison remains vitreous and does not crystallise.

If the sugar is "worked up," i.e. stirred, before it has been sufficiently cooled, the sugar tends to separate in rather larger crystals, and the fondant is likely to be harder, less plastic, and to taste "rough."

On the other hand, if the sugar is over-cooled crystallisation will take place very slowly, and a good deal of difficulty may be experienced in forming the fondant.

On a large scale the working up of the sugar is effected by means of mechanically driven paddles or stirrers, and the fondant, after the addition of flavouring and colouring matters, is passed into small moulds made in corn starch, or in sheets of india-rubber.

Partial crystallisation of the sugar can also be effected by pulling out a plastic mass of hot, boiled sugar over a hook to form a long string or rope. The sugar is then folded and refolded on itself, again drawn out, and these processes repeated until the sugar becomes distinctly fibrous in texture and has a characteristic sheen. Sugar which has been treated in this manner rapidly hardens on keeping, and is frequently sold in bars as some variety of "rock."

Sweetening Power of Sugar.

When sugar is added to fruit as a sweetening agent, the sweet flavour of the sugar masks the sour or acid flavour of the fruit, but the sugar does not in the chemical sense of the word neutralise the acidity of the fruit. The amount of free acid present in the mixture will be the same before and after the addition of the sugar.

Sugar may be added to the fruit and cooked with it, or may be added after cooking and before eating.

If the sugar is cooked with fruit which is distinctly acid, some of the sugar will be inverted by the acid present. The relative sweetening value of sugar added before cooking, as compared with that added after, must evidently depend largely on the comparative sweetness of cane sugar and the invert sugar produced therefrom. This is probably to some extent a matter of individual taste, and it is difficult to obtain any exact measure of the sweetening powers of different substances.

A method for comparing sweetening values has been suggested by Paul ("Chem. Zeit.," 1921, **45**, 705; "Untersuch. Nahre. Genussm," 1922, **43**, 137). Two solutions of the particular substances are prepared, one of which tastes sweeter and the other not so sweet as a standard (e.g. 3 per cent.) solution of cane sugar. Between these extremes further series of solutions of definite concentrations are compared with the standard, and curves from which the sweetening value can be calculated are plotted. For the estimation of sweetening power the average results, as found by 20 to 30 persons, should be taken.

The sweetening value is defined as the number of grams of pure sucrose which must be dissolved in a definite volume of water, to give a solution which tastes as sweet as a solution of 1 gm. of the substance in question in the same volume of water. The sweetening value of sucrose is thus = 1.

The following sweetening values were obtained for the different sugars.

Sucrose = 1. Dextrose = 0·53. Lævulose = 1·05. Lactose = 0·27.

Assuming that the sweetening value of invert sugar may be taken as the mean of that of dextrose and lævulose, i.e. $\frac{0{\cdot}53 + 1{\cdot}05}{2} = 0{\cdot}79$, it would appear that the sweetening value of invert sugar is less than that of sucrose, and that the most efficient method of sweetening fruit would be to add the sugar after cooking.

It is probable that the apparently greater sweetening effect, which in some cases seems to be produced by previously added sugar, may be due to the more complete solution of the sugar in the liquid. When added after cooking, the sugar in a crystalline form is usually sprinkled over the fruit, and the

mixture eaten before the sugar has dissolved. If full advantage is to be derived from the sugar, it should be completely in solution before the mixture is eaten.

Chocolates.

Chocolates are prepared by covering a "centre" made of fondant, or other sweetmeat, with a thin coating of chocolate. This latter substance is a product obtained from the cocoa bean, and differs from cocoa in containing a relatively high proportion of fat in the form of cocoa-butter (see p. 195).

The chocolate is heated to about 88° F., and stirred continuously until it is reduced to a smooth, viscous liquid.

The "centre" is dipped into the chocolate, the excess of liquid drained off, and the sweet placed on a flat surface to cool.

It is essential that the chocolate, which is to be regarded as of the nature of an emulsion, should not be over-heated, and that the setting of the coating should take place as rapidly as possible. If these conditions are not observed, the ingredients of the chocolate tend to separate out in layers, so that on cooling the surface appears dull and streaky instead of glossy and uniform.

The stirring of the chocolate, besides assisting the melting process, also tends to prevent any separation of the emulsion.

The Heating of Milk.

It is a matter of common observation that milk, when heated, forms a film or skin on the surface, and that if this skin is removed another is rapidly formed. It is owing to the formation of this skin that the milk shows a tendency to "boil over."

The skin prevents the free escape of steam, so that the liquid below the skin becomes slightly superheated, and if the heating is continued the milk suddenly froths up.

The formation of this skin has been attributed, in the past, to various causes, e.g. to evaporation at the surface of the liquid, to the coagulation of lactalbumin, and to the separation of fat.

Determinations of the protein content of the successive skins formed, show that the amount of protein which can be

removed from the milk in this manner exceeds the total amount of lactalbumin present, and examination of protein in the skin shows it to be largely composed of casein.

The casein in milk is held in solution in the form of a compound with calcium (calcium caseinogenate). On heating, this compound undergoes hydrolysis, and the casein tends to separate out from solution with the formation of a skin at the surface of the liquid and a granular deposit on the bottom of the containing vessel. As the casein separates, it will tend to entangle some of the fat and carry this to the surface with it. The formation of a coherent film is undoubtedly assisted by the presence of fat, and probably also by surface evaporation. These, however, are to be regarded as contributory factors, the essential factor being the separation of casein from solution by hydrolysis.

Experiments on the Heating of Milk.

1. **To show that a Skin is Formed when Milk is Heated in a Closed Vessel** (i.e. when surface evaporation is reduced to a minimum).

Fill a small thick-walled glass bottle, about two-thirds full, with milk. Close the bottle with a tightly-fitting cork, and secure the cork firmly with string or wire. Heat the bottle carefully in a water bath, raising the temperature slowly. If heated too rapidly the bottle may crack. After heating for some time, a skin will form. The skin can be seen most readily by tilting the bottle sideways so that part of the surface is covered by, and part of the surface is free from, skin.

To show that the formation of the skin is not due to surface oxidation, carbon dioxide gas should be bubbled through the milk in the bottle for a few minutes to replace the air, the cork then quickly replaced and the heating in the water bath repeated. A skin will again be formed.

2. **To show that the Formation of a Skin is not Dependant on the Presence of Fat.**

For this purpose a 3 per cent. solution of casein in lime water should be prepared so that the concentration of the casein in the solution will be approximately the same, as that in milk (see p. 1).

Measure out 100 c.c. of lime water into a 350 to 500 c.c. stoppered bottle, and weigh out 3 gms. of casein on a watch glass. By means of a spatula, transfer a small portion of the casein to the lime water in the bottle, insert the stopper and shake well. When the casein has dissolved to form an opalescent solution, add more casein and repeat the process until all the casein has been dissolved. Samples of commercial casein vary considerably in the ease with which they dissolve in lime water, but in most cases it is possible, with a little perseverance, to bring the casein into solution in this manner. If a small proportion of the casein remains undissolved, it may be disregarded, and the solution may be decanted and used.

Heat some of the casein solution in a glass dish on a water bath and observe that a skin is formed, and also that a granular deposit is left on the bottom of the dish when the solution is poured off.* If a porcelain dish is used, it is more difficult to see the skin which, in this case, is rather transparent.

3. To show that a Solution of Egg-albumin in Lime Water forms a Skin on Heating.

The formation of a skin is not peculiar to solutions of casein, but is also exhibited by solutions of other proteins in lime water. A solution of egg-albumin in lime water can be conveniently prepared from the dried albumin.

About 3 gms. of dried egg-albumin should be steeped for some hours, or over-night, in a small quantity of water, and the solution thus obtained diluted to 100 c.c. with lime water. Heat some of the solution in a glass dish on a water bath, and observe that a skin is formed.

4. To Compare the Solubility in Lime Water of the Skins formed on Heating Milk to Different Temperatures.

Suspend a thermometer in some milk contained in a glass dish or beaker and heat on a water bath. Note the temperature at which a skin first begins to form. This is usually between 40° C. and 50° C., but varies to some extent with the rate at which the temperature of the milk rises. Also, after

* When lime water alone is heated for some time a slight scum is formed, due to the formation of calcium carbonate by the absorption of carbon dioxide from the air. This is, however, quite different in character from the coherent film formed by the casein solution.

experience has been gained the presence of a skin can be detected more easily than at first.

Keep the milk at the temperature at which the skin first begins to form, for some little time, until a skin of appreciable thickness is obtained.

Then, with a glass rod, transfer the skin to a test tube, add lime water, and shake gently. The skin will dissolve to form an opalescent solution.

Continue the heating of the milk until the temperature rises to 70° C. Remove the skin which is formed at this temperature and shake with lime water as before. The skin formed at the higher temperature does not dissolve in the lime water. These experiments show that at the lower temperature the casein is liberated in a form in which it will redissolve in lime water, but when the temperature is raised to 70° C. the casein liberated is no longer soluble in lime water. Similar alterations in the solubility of the casein can be observed when solutions of casein in lime water are heated.

5. To compare the Changes which take place in Solutions of Casein in Lime Water on Heating to Different Temperatures.

The 3 per cent. solution of casein used in (2) above, is usually slightly turbid, and the changes which take place on heating can be seen more readily if a more dilute solution is used.

Dilute some of the 3 per cent. casein solution 1 : 10 with lime water, and heat a portion of this diluted solution, in a test tube, to 40° C. on a water bath. Remove the solution from the water bath and compare its appearance with that of some of the unheated solution contained in another test tube.

The heated solution, it will be observed, is more turbid than the cold solution, but as the solution cools this turbidity decreases, and the solution finally regains its original appearance.

Again heat the solution, this time to a temperature of 70° C. The solution again becomes turbid, but this turbidity does not clear on cooling as in the previous case.

Thus the hydrolysis of the casein compound is reversible if the temperature is not raised much above 40° C., and the reaction may be represented thus :—

Calcium caseinogenate + $2H_2O \rightleftarrows Ca(OH)_2$ + casein.

On the other hand, if the solution is heated to 70° C., or much above 60° C., the reaction is no longer reversible, owing to the conversion of the casein at this temperature to a form in which it is insoluble in lime water.

If milk is heated at the boiling-point for some time, further changes take place, and the protein undergoes slight decomposition, as is shown by the liberation of small amounts of hydrogen sulphide.

6. To show that Milk Liberates Hydrogen Sulphide on Heating.

Introduce about 100 c.c. of milk into a round, flat-bottomed flask of about 350-500 c.c. capacity. Plug the neck of the flask with cotton wool and down one side insert a piece of moistened blue litmus paper, and down the other a piece of filter paper moistened with lead acetate solution. Heat the flask on a gauze over a burner, and keep the temperature of the milk as near boiling-point as possible, without allowing the milk to froth up the neck of the flask. After a time the lead-acetate paper will turn brown, and the litmus paper red.

The liberation of the volatile sulphide is facilitated by the presence of alkali, and the reaction may be hastened by adding a little sodium bicarbonate to the milk. In this case it will be noted that the milk becomes brown on heating, this change of colour being due to a reaction between the casein and the alkali.

The brown colour produced in "soda cakes," i.e. cakes in which sodium bicarbonate alone, and not a mixture of sodium bicarbonate and acid, is used as the raising agent (see p. 127), is to be attributed to the same reaction.

COOKING OF VEGETABLES.

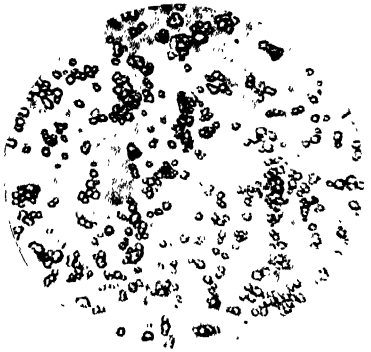
As already stated, the chief processes involved in the cooking of vegetables are the softening and breaking down of the cellular tissue of the plant, which is largely composed of cellulose, and the swelling and gelatinisation of any starchy granules present. These changes are usually effected by exposing the vegetable to the action of boiling water or steam.

As soon as the cell walls begin to soften and weaken, the water will be able to penetrate into the cells and mingle freely with the cell contents. In this manner the starch granules

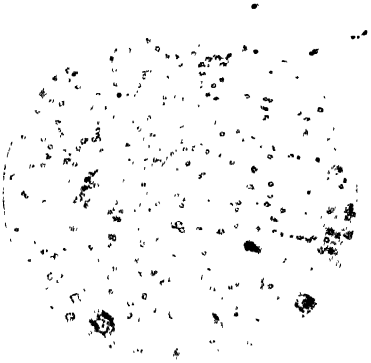
PLATE III

MICROPHOTOGRAPHS

(Magnification—approximately 240)



A. Rice starch

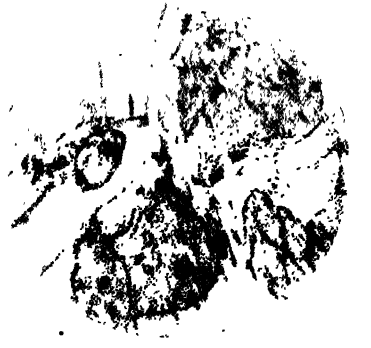


B. Pepper

C. Raw potato

D. Partly cooked potato (i)

E. Partly cooked potato (ii)



F. Cooked potato

[*To face p.* 23

will be brought into contact with the boiling water; these will tend to swell and burst, and in so doing will assist in the further disintegration of the cell walls. These stages in cooking may be followed out in the case of a potato by examining thin sections of raw, partially-cooked, and cooked potato under the microscope.

Examination of Raw, Partly-cooked, and Cooked Potato.

Cut a potato in half, and with a sharp knife or razor, slice off a number of thin sections. Select the thinnest portions of these sections and prepare several slides, mounting in water and covering with a cover slip. Examine these slides under the microscope. A good section will show a network of cellular structure surrounding groups of starch granules which, from their characteristic shape and appearance, can be readily identified as potato starch (see Plates II. and III., also p. 101). Heat one-half of the potato in boiling water for about ten minutes, and when cool cut and mount sections as before. On carefully examining these sections, portions which show breaks in the cell walls can usually be found, and also starch granules which appear ill-defined in outline or which show other more marked signs of swelling (see Plate III., *D* and *E*). In *E* the cooking process has been carried further than in *D*.

Heat the other half of the potato in boiling water until cooked, and then allow to cool. It is difficult, especially if the potato is at all "floury," to cut satisfactory sections of the cooked potato, but with a little care it is usually possible to flake off small portions which will adhere together sufficiently well to be mounted on a slide.

On examining these under the microscope, it will be found that individual starch grains can no longer be distinguished, and that the network of cellular structure has disappeared (see Plate III., *F*).

The Cooking of Green Vegetables.

A good deal of experimental work in connection with the cooking of vegetables has been carried out in these laboratories, and a short account of some of the more important results obtained with illustrative experiments may be given here.

For further information, see Masters, "Biochem. J.," 1918, **12**, 231, and Masters and Garbutt, "Biochem. J.," 1920, **14**, 76.

Colour Changes Produced in Green Vegetables on Cooking.

As no method of cooking green vegetables can be considered satisfactory from the practical point of view, unless the green colour of the vegetables is preserved, some study may be made of the changes in colour produced when green vegetables are cooked under various conditions.

It appears to be fairly generally accepted that the preservation of the colour of green vegetables during cooking can best be effected in the following ways :—

(1) By cooking in a considerable volume of rapidly boiling water in an open vessel, i.e. in a vessel without a lid.

(2) By the addition of a small amount of alkali, usually sodium bicarbonate, to the cooking water.

Experiments in illustration may be carried out as follows :—

Cook portions of cabbage, or other green vegetable, in the following ways :—

(1) Boiling tap water, in an open vessel.

(2) Boiling distilled water, in an open vessel.

(3) Boiling tap water, to which a little sodium bicarbonate has been added (about 0·5 gm. per litre), in an open vessel.

(4) Boiling tap water, made slightly acid with acetic acid, in an open vessel.

(5) Boiling tap water, in a saucepan closed with a tightly-fitting lid.

In each case strain off the vegetables when cooked, and compare the colours of the different portions and also that of the corresponding cooking waters. Test the reaction of the cooking waters 1, 2, and 5 with litmus paper.

The addition of alkali to the water has the effect of producing a much brighter green colour than is observed in the other cases, and it will also be noted that the cooking water is distinctly coloured. The addition of acid, on the other hand, has the reverse effect, the green colour is changed to a brownish shade and the cooking water is only very slightly coloured.

Chlorophyll, of which the green colouring of the vegetable is composed, is soluble in alkaline solutions, and on the death of the plant cell the colour changes to a brownish-green (formation of chlorophyllan), unless alkali is present.

On extracting green vegetables with alcohol, a dark-green extract is obtained. The colour of this solution deepens on the addition of alkali, and rapidly changes to brown on the addition of acetic acid. Thus, in order to preserve the green

colour of the vegetables, the presence of any acid substance should be avoided. The dark-coloured cooking water observed when sodium bicarbonate was used, is to be attributed to the solubility of the chlorophyll in the alkaline solution.

The vegetables cooked in tap water (London), are usually quite a good colour, whilst those cooked in distilled water are not, and the colour of the latter is usually only slightly better than that of those cooked in water containing acid. In the two former cases the cooking waters will probably be found to be slightly acid to litmus, and it will be noted that the cooking water is more coloured in the case of the tap water than in the case of the distilled water. It should be remembered that London tap water is slightly alkaline. The differences in behaviour of the tap and distilled water are to be attributed to this alkalinity.

The vegetables cooked in the closed pan will be found to be distinctly inferior in colour to those cooked in an open vessel, and the cooking water, as before, is acid.

The action of acid on the chlorophyll, taken in conjunction with the fact that the water in which the vegetables have been cooked has an acid reaction, although the tap water was originally alkaline, suggests that acid substances may be produced from the vegetables on cooking. Such acids, if volatile in steam would, to a great extent, be removed if the vegetables were cooked in an open vessel in rapidly boiling water, but would be mostly retained if a covered vessel were used. This would afford an explanation of the differences in colour observed with vegetables cooked in open and in covered pans.

Some examination of the volatile products produced during the cooking of vegetables may, therefore, next be made.

Examination of the Volatile Products Produced in Cooking Vegetables.

Heat a portion of the vegetable in boiling water in a round bottomed flask connected with a condenser and collect the distillate. Test portions of the distillate as follows :—

(1) Add a few drops of litmus solution. An acid reaction is obtained, showing that volatile acids have been liberated.

(2) Add a few drops of lead acetate solution ; a dark-brown colour is produced, showing the presence of sulphide in the distillate.

(3) Make the solution slightly alkaline with ammonia and

then add a few drops of a dilute solution of sodium nitroprusside. A violet coloration is produced, thus confirming the presence of sulphide. These results show that acid and sulphide are liberated, but give no indication as whether or not any volatile organic acids are also liberated.

Some experiments made in this connection showed that if carbon dioxide was bubbled through the distillate, until all the volatile sulphide was removed, and the carbon dioxide was then also removed by boiling the solution for half an hour under a reflux condenser, the solution was still slightly acid. Further, some indication that formic acid and acetic acid were present was also obtained, although it is difficult to make conclusive qualitative tests in dealing with such small quantities.

Losses in Solid Matter during Cooking.

The average results obtained for a number of experiments show that from 30 to 40 per cent. of the total solid matter present in the vegetable is extracted and found in the cooking water when the vegetable is cooked in boiling water. This loss is reduced by about 5 to 10 per cent. when alkali is added to the water, since the addition of alkali reduces the time required for cooking.

The cell walls of the plant are composed of a hydrated form of cellulose which is more or less soluble in alkaline solutions. The alkali added thus assists the softening or cooking process, so that the time required for cooking is reduced; and it may be taken as a general rule that the proportion of solid matter extracted increases with the time of cooking.

This statement does not, however, apply in cases where vegetables are cooked under conditions which are likely to cause complete disintegration. Thus, if excess of alkali is added to the cooking water, the vegetables rapidly soften, and if the cooking is continued the vegetables are reduced in a short time to a pulp composed of solid matter and water.

The addition of salt to the cooking water has no marked effect, either on the loss of solids or on the time of cooking. If vegetables are cooked by steaming, only about 9 to 10 per cent. of the total solid matter is extracted. This method of cooking necessitates, of course, the use of a closed vessel, in consequence of which, the cooked vegetables have a bad colour, and the time of cooking is increased.

The results of the foregoing experiments suggest that

this difficulty could be obviated if the steam could be rendered alkaline. This can be effected by steaming the vegetables over boiling water, to which a little ammonium carbonate has been added.

The Steaming of Vegetables Over Water Containing Ammonium Carbonate.

Take two portions of green vegetable, each weighing 100 gms. Cook each portion in a steamer over 1 litre of boiling water, and in one case add 0·5 to 1·0 gm. of ammonium carbonate to the water. Note the time required for cooking in each case, also the colour and odour of the vegetable.

It will be observed that the addition of ammonium carbonate improves the colour, and also reduces considerably the time of cooking, and that the cooked vegetable does not smell of ammonia. Determinations made of the losses on cooking show that the proportion of solid matter extracted by steaming is reduced (average total loss about 5 per cent.) when ammonium carbonate is used. This reduction may be attributed, as previously suggested, to the reduced time of cooking.

This method of steaming may also be used with advantage for cooking other vegetables, e.g. potatoes, carrots, etc. It is found that the observations made as regards loss of solids and reduced time of cooking apply also in the case of these vegetables.

The Cooking of Dried Legumes (Beans and Peas).

The time required for cooking dried legumes is considerably greater than that required for cooking most of the other varieties of vegetable, and a number of different methods for reducing the time of cooking have been suggested.

Thus it is a usual practice to soak these vegetables in water over-night, and the use of soft water for cooking is also frequently advocated.

To test the value of some of these suggestions, the following experiments may be made :—

Weigh out four 100 gm. portions of dried haricot- or butter-beans, and treat as follows :—

(1) Soak in 250 c.c. of tap water over-night, or for not less than four hours.

(2) Soak in 250 c.c. of distilled water over-night, or for not less than four hours.

(3) Soak in 250 c.c. of tap water containing 2·5 gms. of sodium bicarbonate over-night, or for not less than four hours.

(4) To be cooked without previous soaking.

Strain off the water in each case and cook (1) in 400 c.c. of tap water, (2) in 400 c.c. of distilled water, (3) in 400 c.c. of tap water, and (4) in 500 c.c. of tap water.

It will be found that the soaked beans have absorbed about 100 c.c. of water, hence an additional 100 c.c. is allowed in cooking the unsoaked beans in order that the amount of water used for cooking may be the same in each case.

Add each portion of beans to the measured volume of cold water contained in a saucepan. The saucepans should all be of the same size and shape.

Cover each saucepan with a lid, note the time, raise the temperature of the water to the boiling-point, and keep the water boiling fairly rapidly, and at the same rate in each case, until the vegetables are cooked. Note the time taken in each case. It will be found that soaking the beans before cooking, either in tap or distilled water, does not reduce the time of cooking to any marked extent. Those soaked and cooked in distilled water may cook rather more rapidly than those in tap water, but the difference in time is usually not very great, and too much reliance should not be placed on the result of one experiment. Since time does not usually allow of the repetition of such experiments, it is suggested that students should record not only their own results, but also the average given by the results obtained from all the different members of the class, excluding, of course, any which are obviously inaccurate.

The greatest difference in time will be observed in the case of the beans which were soaked in the water containing sodium bicarbonate. These usually cook in about half the time required in the other cases.

Determinations which have been made of the loss of solids on cooking show that in this case also decrease in the time of cooking is accompanied by decrease in the proportion of solid matter extracted during cooking.

Experiments were also made to study the effect of adding sodium bicarbonate to the cooking water, instead of to that used for soaking. The results of these experiments showed that if the beans were cooked in water containing

more than 0·25 per cent. of sodium bicarbonate, they became yellowish in colour, softened and disintegrated so rapidly that a large proportion of the solid matter passed into the cooking water. The loss in solid matter was therefore very high. If the proportion of sodium bicarbonate was reduced below 0·1 per cent. there was no appreciable reduction in the time of cooking.

Thus better results can be obtained by adding the sodium bicarbonate to the soaking water, than by adding it to the cooking water.

If excess of sodium bicarbonate is added to the soaking water the beans tend to exhibit, though in a less degree, the features observed in the case of beans cooked in water containing over 0·2 per cent. of sodium bicarbonate, and the loss in solid matter during cooking increases. Careful experiments show that, contrary to statements sometimes made in cookery books and elsewhere, the addition of salt to the cooking water does not appreciably increase the time of cooking. The flavour of the vegetables is, of course, much improved by the addition of salt. The most satisfactory results were obtained when about 0·25 per cent. of salt was added to the water, the flavour being considered rather too pronounced when larger quantities were used. The addition of the salt also tends to reduce slightly the proportion of solid matter extracted during cooking.

Peas.—It should be noted that in the case of dried peas, soaking in water containing sodium bicarbonate, previous to cooking, has an even more marked effect than that observed in the case of the beans. The presence of the alkali assists, as in the case of other green vegetables, to preserve the colour, and the peas should be cooked in an open pan.

In cooking on a large scale it is often more convenient to cook vegetables, which require a considerable time for cooking, in steamers rather than in boiling water. If the beans (or peas) are placed in a vessel, and sufficient water added to cover them, they will cook satisfactorily in a steamer, and the proportion of solid matter extracted will be less than when the vegetables are cooked in boiling water.

Here, again, time of cooking can be considerably reduced by soaking in water containing 1 per cent. of sodium bicarbonate, and also by rendering the steam alkaline by the addition of ammonia.

(Further details are given in the papers referred to above.)

BREADMAKING.

Bread, as ordinarily understood, is the product obtained on baking a dough of flour and water, which has been aerated by the liberation of carbon dioxide gas in the mixture. The gas can be generated by chemical raising agents, the nature and mode of action of which have already been described (see p. 126), or may, as in the case of so-called "aerated bread," be introduced by specially devised mechanical processes; but the *fermentation process*, in which the gas is produced by the alcoholic fermentation of sugar by yeast, is still almost universally employed. The flavour and texture of the bread prepared in this manner are generally considered to be superior to that obtainable by any other process.

Preparation of Bread by the Fermentation Process.

The art of making bread by the fermentation process dates back to a very early period, and has been practised all the world over for centuries past. Different countries and districts have evolved methods which are adapted to meet their special needs, and the nature of the raw materials available. All that can be attempted here is to give a brief outline of the fundamental processes involved.* Even these fundamental processes, although they have formed the subject of numerous scientific investigations, are as yet by no means completely understood. Slight differences in the properties of the flour, which may be of such a character that they cannot be detected by chemical methods, may considerably modify the character of the loaf produced. So that whilst the importance of certain factors in evaluating a flour has now been established, baking tests still form the only reliable method of judging the bread-making properties of flour.

Nature of Yeast.—In this country the yeast (see p. 105) used for bread-making is usually distiller's yeast, or compressed yeast which has been prepared from distiller's yeast, but brewer's yeast is also sometimes used, more particularly in the preparation of what is known as "farmhouse" bread. Yeast, it should be remembered (see p. 106), contains the enzymes invertase and zymase, and is therefore able to

* For further information reference should be made to "The Technology of Breadmaking," by Jago (see p. 268).

bring about the inversion of cane sugar, and the conversion of the dextrose and lævulose thus formed to alcohol and carbon dioxide.

Preparation of the Dough.—The yeast is mixed with flour, water, a little salt, and some form of yeast food, e.g. malt extract, cane sugar, etc., may be added, but this is not always necessary.

The dough is well kneaded to ensure even distribution of the ingredients, and is then set aside in a warm place to rise

Fermentation of the Dough.—The yeast rapidly sets up alcoholic fermentation, i.e. the decomposition of the sugar into alcohol and carbon dioxide gas. The gas, which is to a large extent retained in the dough, causes the dough to distend or "rise." This liberation of carbon dioxide may be regarded as the essential function of the yeast, but as the fermentation proceeds, slight, though important, changes take place in the properties of the dough.

The proteins in the flour undergo changes which are similar to, if not identical with, the earlier stages of digestion. Peptones are formed and the gluten (see p. 120) becomes softer and, within certain limits, more elastic, but if the fermentation is allowed to proceed for too long the gluten softens still further, and its peculiar elasticity in a great part disappears. It is uncertain to what extent these changes in the gluten are due to the specific action of the yeast, as they occur to some extent, although much more slowly, when the flour is mixed with water only.

Under the action of yeast also, the albuminous bodies in the flour acquire the power of effecting the hydrolysis of starch with the consequent production of dextrin and maltose.

Further changes may be brought about by micro-organisms other than yeast, which may be present in the flour, or which may have been introduced with the yeast. For example, by the action of lactic acid forming bacteria some of the sugar may be converted to lactic acid, and the acid thus produced tends to soften and dissolve the gluten. The time allowed for the fermentation of the dough depends on the character of the flour and on the method which is to be employed for making the bread.

Behaviour of Strong and Weak Flours.—Flour which is to be used for making bread by the fermentation process should, on the addition of water only, yield sugar in such proportion that the gas obtained therefrom by fermentation will suffice

for the inflation of the dough. Further, the nature and quantity of the gluten should be such that it is capable of retaining sufficient gas in the dough, and elastic enough to allow of the uniform distention of the dough by the gas. . . .

A flour which has these properties is described as a *strong* flour (see also p. 120). The gluten in a strong flour may be firm and rather inelastic to start with, but as the fermentation proceeds the gluten will, as explained above, become softer and more elastic. Hence the properties of the gluten are improved during the fermentation process, and the dough may safely be allowed to ferment and rise for a considerable period.

In the case of a weak flour, on the other hand, in which the gluten is already soft, the further softening brought about during fermentation is liable, if the fermentation is allowed to proceed for more than a short period, to destroy the gas-retaining properties of the gluten. In consequence, the dough is likely to inflate rapidly and then collapse.

If the fermentation process is used for making wholemeal bread, the introduction of the bran tends to bring about a marked hydrolysis of the starch during fermentation, so that the dough becomes soft and clammy. On this account for making wholemeal bread a chemical raising agent is often used in preference to the fermentation process (see p. 131). When the fermentation process is used it is customary to make a "sponge" of a strong flour and mix the wholemeal flour with the dough at a later stage (see below).

Breadmaking Processes.

In some processes of bread-making, e.g. "*sponge process*," only a portion of the flour is mixed with the yeast in the first stage. This dough or *sponge* is set aside for several hours to rise, the remainder of the flour is then mixed in, and the dough, after again standing, this time for a considerably shorter period, is ready for baking.

The advantages of this method are that a relatively small quantity of yeast is required, and also that it is possible by this process to obtain good results with blends of strong and weak flours. The "sponge" is made with a strong flour and a proportion of weak flour, which is often superior in flavour to the strong flour, is mixed in with the sponge in the later stage.

If the whole of the flour is mixed directly with yeast as, for example, in the "*off-hand*" or "*straight dough*" process, the bread can be prepared in a shorter time, but the flour must be blended before mixing with the yeast, and a relatively large quantity of the latter is required.

In this case, in order to obtain the maximum effect from the yeast, and so reduce the quantity required, the dough, after it has risen for some time, is again well kneaded: The kneading, by introducing fresh air into the dough, tends to restore the vital activity of the yeast. The dough is then set aside to rise for a second period, and afterwards baked.

Baking of the Dough.—The dough, after fermentation, is baked in an oven at a temperature of about 450° to 500° F.

As the temperature of the dough rises the gases therein expand, and the dough rapidly swells. The outside of the dough becomes coated with a *crust.* The starch on the surface is hydrolysed to dextrin and sugar, and this at the temperature of the oven becomes partially caramelised, and so gives the crust its characteristic colour and flavour.

The effect of heat on the interior of the loaf is to evaporate a portion of the water, and also to expel the carbon dioxide and part of the alcohol produced during fermentation

Partial gelatinisation of the starch also occurs, but the water present in the dough is insufficient to bring about complete gelatinisation of the starch.

Baked bread still contains about 40 per cent. of water.

Flour Improvers.

Mineral salts and other substances which are added to flour with a view to improving the colour, texture, or size of the loaf obtainable from a given weight of flour, are usually described as *flour improvers.** These substances appear to act chiefly in the direction of modifying the changes which take place during fermentation. In some cases, e.g. malt extract and phosphates, they may be regarded as yeast foods or stimulants which tend to increase the supply of gas produced during fermentation. In other cases, however, more particularly the mineral salts, it is the physical properties of the gluten which appear to be mainly affected.

* See Local Government Board Food Reports, No. 12, H.M. Stationery Office, 1911.

It has previously been noted that the strength of a flour is chiefly dependent on the quantity and physical character of the gluten, and it is now generally accepted that the presence or absence of certain mineral salts, particularly soluble phosphates, is an important factor in determining the strength or weakness of the flour. These salts appear to exert a binding effect on the gluten, and also to prevent the gluten from softening unduly when exposed to action of yeast during fermentation.

The principal salts used as flour improvers are the soluble phosphates (i.e. the phosphates of sodium and acid calcium phosphate), calcium sulphate, alum, and persulphates. Also the common salt, ordinarily added in bread-making for flavouring purposes, may be regarded as acting to some extent as an "improver."

In the case of flours which show a tendency to become acid or "sour," the use of lime water for mixing the dough has in the past been advocated.

In practice, however, the results obtained are unsatisfactory, and the use of lime water has now been largely discontinued. In this connection some experiments were made in these laboratories with "Government controlled" war-flour, which showed a marked tendency to become sour. (H. Masters and M. Maughan, "Biochem. J.," 1920, **14**, 586.) It was found that the loaves made with lime water were always smaller than their controls (i.e. loaves similarly prepared, but mixed with water instead of lime water). Also, that the doughs mixed with lime water appeared drier than the doughs mixed with water, and that the former, whilst rising, formed a thick outer skin.

Determinations of the acidity of the freshly-baked bread and of the bread after keeping several days showed that whilst the lime water neutralised any acidity in the flour, it did not prevent the production of acid on keeping.

It should also be noted that in the course of these experiments the effect of a number of different substances as flour improvers was tried, and although some of these had an appreciable effect in increasing the volume of the loaf obtained when the bread was prepared by the fermentation process, in no case was any alteration in the size of the loaf observed when the bread was raised with a chemical raising agent.

Baking Tests.

If suitable utensils and oven are available, baking tests can be carried out on a small scale in the laboratory, and with a little practice in manipulation, consistent results can be obtained.

Such experiments, however, should be only attempted by those who have some practical knowledge of the art of bread-making, and it is again necessary to emphasise that deductions should not be made from the results of one experiment only. Baking tests may be made to compare size (i.e. volume) of the loaf obtained from equal weights of different flours, or to test the effect on the size of the loaf of the addition of some substance to the flour. Doughs containing equal weights of all the essential ingredients, i.e. flour, yeast, water, and salt, are prepared so that the doughs differ only in the nature of the flour used, or are prepared with the same flour, with and without the addition of some substance, the effect of which on the bread-making properties of the flour it is desired to test.

The doughs should be subjected to the same treatment throughout and baked in the same oven, at the same temperature and in tins of the same size and shape.

Either cylindrical or straight-sided rectangular tins should be selected, so that the volume of the loaf can be approximately calculated from its dimensions.

Then, since the tins have bases of equal area, the height of the loaf will be the only variant, and the volumes of the loaves will vary in proportion to their heights.

To obtain the approximate height of the loaf, the loaf should be cut through the centre with a sharp knife, the greatest and least heights measured, and the mean taken.

The *volume* of the loaf may be calculated in the ordinary way. It is equal to the area of the base *multiplied by* the height. In the case of a loaf baked in a cylindrical tin, the volume $= \pi r^2 h$, where r = the radius, and h = the mean height. $\pi = \frac{22}{7}$.

Details as to the quantities of ingredients, method of mixing, etc., which may be used for small scale baking experiments, are described in the paper referred to above, and these may be briefly summarised here.

Directions for Small Scale Baking Tests.

The essential ingredients may conveniently be mixed for each loaf in the following proportions :—

(1) 200 gms. of flour.
(2) 5 gms. of yeast.
(3) 2 gms. of sugar (to act as yeast food).
(4) 2 gms. of salt.
(5) Liquid for mixing 95 to 120 c.c.

The volume of liquid, usually tap-water, required for mixing the dough to the necessary consistency varies with the nature of the flour, and the total volume used should include any liquid or solution added for the purpose of the experiment. For this quantity of dough a cylindrical baking tin having a diameter of about 10 cm. and depth of about 7 cm. is convenient in size and shape.

Method of Mixing.—The yeast is mixed to a thin cream with the sugar, and then added to the flour and salt. The liquid is next mixed in, and the dough thus formed kneaded for about five minutes.

First Rise.—The dough is now allowed to rise in a warm place or in an oven kept at about 25° C., until the surface skin cracks. The time required for rising will vary with the nature of the flour, and is usually from forty-five minutes to one hour.

Second Rise.—The dough is again kneaded, then placed in a greased baking tin, and set aside to rise, at 25° C., until it has about doubled its size. The time necessary for this second rise varies with the flour in the same manner as for the first.

Baking of the Dough.—The dough is put into a hot oven, the temperature raised for the first ten minutes, and then decreased at intervals until the bread is cooked.

It is difficult and often misleading to give exact data for the regulation of the temperature and time of cooking, since much will depend on the type of oven and method of heating employed. Thus, for example, in a gas-heated oven the conditions prevailing are different from those in an electrically-heated oven, and if cooking operations are carried out in gas and electrically heated ovens, heated to the same temperature as registered by thermometers, it is by no means certain that

similar results will be obtained, or that the time required for cooking will be the same in the two cases.

For experimental purposes an electrically-heated oven is preferable, since required conditions of temperature can be reproduced by timing, and the temperatures can thus be more easily controlled.

In making comparative experiments it is only necessary that the same conditions of preparation and baking should be preserved throughout. Hence, in the case of loaves which are prepared with the same sample of yeast under similar conditions, and which are baked in the same oven for the same length of time, a fair comparison should be obtained.

In cases, however, where it is desired to compare the results with those obtained in another series of experiments, made at another time, a *control* loaf made from the same ingredients as those used in the previous experiments should be prepared and cooked as one of the batch. The volumes of the other loaves can then be compared with and calculated relatively to the control. In this manner possible errors due to variations in the properties of the yeast, or to conditions of mixing and baking, can to a great extent be eliminated.

A convenient method is to take the volume of the control loaf in each case as 1000, and to calculate the volumes of the other loaves relatively to this.

For example, if the volume of the control loaf were 559·8 c.c. that of one of the other loaves of the batch 589·3 c.c., then taking 559·8 c.c. as equivalent to 1000, 589·3 will be equivalent to

$$\frac{1000}{559{\cdot}8} \times 589{\cdot}3 = 1053.$$

FLAVOURING AGENTS.

In the preparation of cooked food, small quantities of flavouring agents, condiments, etc., are usually added. The peculiar flavouring qualities of many substances which have a well-defined and characteristic flavour can be traced to the presence of small quantities of constituents which possess the particular taste and odour in a marked degree.

These flavouring matters have in many cases been isolated and obtained in a state of purity. In a large number of instances their *physical properties* are those of a volatile oil,

They are liquid, more or less oily in their nature, evolve a distinct and often powerful odour at ordinary temperatures, but boil at a much lower temperature than the glycerides or *fixed oils*. Such bodies are described as *essential oils*.

Essential Oils.—Essential oils are not composed of glycerides, but many of them are of the nature of aldehydes, e.g. oil of almonds (benzaldehyde),

$$C_6H_5CHO \text{ and citral } C_9H_{15}CHO.$$

Physical and chemical methods, such as are ordinarily used in organic chemistry, are largely relied on for checking the purity of such products.

Thus the methods used for their examination involve determinations of specific gravity, boiling-point, and in some cases specific rotatory power, and the formation of characteristic derivatives.

Almond Oil and Essential Oil of Almonds or Oil of Bitter Almonds.—As confusion occurs sometimes as to the nature of the different "*oils*" which can be obtained from almonds, a brief description of these products may be given here. Almonds (both sweet and bitter) contain a *fixed non-volatile oil*, which can be expressed from the nut or kernel and is sold as *almond oil*. On extraction with water and distillation in steam, crushed bitter almonds, after the removal of the fixed oil, yield a *volatile essential oil*.

This essential oil does not exist as such in the almond, but results from the action of water on the glucoside amygdalin under the influence of the enzyme *emulsin*. Both the glucoside and the enzyme are present in the almond, and are brought into contact with one another when the almonds are crushed and treated with water.

$$\underset{\text{Amygdalin.}}{C_{20}H_{27}NO_{11}} + 2H_2O \rightarrow \underset{\text{Benzaldehyde.}}{C_6H_5CHO} + \underset{\text{Glucose.}}{2C_6H_{12}O_6} + \underset{\text{Prussic acid.}}{HCN}$$

The *essential oil of almonds* thus obtained contains benzaldehyde and also a considerable amount of prussic acid which is a very poisonous substance. This natural oil is a commercial article, and is known as (*essential*) *oil of almonds* or *oil of bitter almonds*, but much of the natural oil is deprived of its prussic acid before being put on the market.

Kernels of apricot and peach yield essential oils almost identical with that obtained from almonds, and a good deal

of the commercial oil is obtained from apricot kernels. Benzaldehyde can also easily be prepared synthetically, and this artificial oil can be used as substitute for, or as an adulterant of, the natural oil.

Oil of *mirbane*, or nitrobenzene $C_6H_5NO_2$, is another adulterant of oil of bitter almonds, but owing to its toxic action it should not be used in foods.

Essential Oil of Lemon.—Oil of lemon consists principally of hydrocarbons, mostly *terpenes*, the most important of which is the terpene *limonene* ($C_{10}H_{16}$). As flavouring agents the terpenes are of comparatively little value, the essential flavouring matter being an aldehyde known as *citral* (see p. 252). For some purposes the presence of the terpenes is considered an objection, and so-called terpeneless oils are now sold in which all or part of the terpenes have been removed, and the citral and other flavouring ingredients alone remain.

Oil of lemon is not the only source of citral. *Verbena*, sometimes called *lemon plant* or *lemon grass*, yields oils containing a high percentage of citral, and consequently lemon grass forms a comparatively cheap source of citral.

Oil of lemon contains odorous constituents other than citral which are not furnished by lemon grass oil. Conversely, lemon grass oil contains odorous and flavouring matters which are foreign to oil of lemon. The presence of lemon grass oil is revealed by the odour of *verbena*, which can be fairly readily detected by an expert.

Essences or Flavouring Extracts.

Many essences or extracts are solutions of essential oils or other flavouring ingredients in alcohol.

Lemon essence or *lemon extract* is an alcoholic solution of oil of lemon containing the colouring matter of lemon peel.

Vanilla essence is a dilute alcoholic extract of the vanilla bean, the flavour of which is due to *vanillin*,

$$C_6H_3(OCH_3)(OH)CHO,$$

the methyl ether of protocatechuic aldehyde.

Vanillin can be prepared synthetically by the oxidation of eugenol, which is the essential constituent of *oil of cloves*.

Synthetic vanillin forms a cheap substitute for vanilla, and on this account is somewhat extensively used. It is doubtful, however, whether for the most delicate flavouring purposes it can be considered as a complete substitute for true vanilla. For although vanillin is the predominant flavouring ingredient of vanilla, it is probable that there are traces of other flavouring matters in the bean or pod, which will be lacking in artificial or synthetic vanillin.

Fruit Essences.—Nearly all fruits possess distinctive flavours, and genuine *fruit essences* can be prepared by obtaining the substances to which the flavour is due in a more or less concentrated form.

In many cases, however, it is difficult to prepare, from the fruits themselves, an extract sufficiently concentrated to give the distinctive fruit flavour when used in moderate quantities. Hence artificial fruit essences, which are made up of esters in varying combinations and proportions to imitate more or less closely various fruit flavours, are substituted for natural products.

In the case of some fruits it has been possible to trace the flavour to the presence of certain specific esters.

Thus the flavour of pineapple is to be attributed to ethyl butyrate, that of quince to ethyl pelargonate, and that of jargonelle pears to amyl acetate. These esters can be prepared synthetically, and are used in the production of artificial fruit essences.

For further information reference should be made to "Foods and Drugs," by Parry (see p. 268), and "Chemistry of Essential Oils and Artificial Perfumes," by Parry (Scott, Greenwood & Son).

Condiments.

Pepper is the dried berry of the pepper plant, and is sold in two varieties, *black* and *white*. The former is obtained from the whole seed, and the latter from the decorticated seed.

Pepper, in addition to a considerable quantity of pepper starch, contains in small quantities a hot pungent resin, an essential oil and an alkaloid *piperine*.

The quality of a pepper depends almost entirely on the amount of resin and alkaloid, although the flavour is influenced by the amount of essential oil. The adulteration of pepper with mineral matter can be detected by high ash value, and

also by shaking with chloroform in a separating funnel. Added mineral matter with a small quantity of natural husk material rapidly sinks to the bottom, and may be drawn off and examined. (Compare "mineral matter in flour," p. 123). Pepper may also be adulterated with ground rice or other starchy matter. The presence of starch other than pepper starch can be detected by a microscopical examination (see Plate III., *B*, p. 237, for pepper starch). In the case of adulteration with starch, the ash value will be low.

Mustard is the ground and sifted seed of the mustard plant.

The pungency of mustard does not depend on the existence of any compound originally present in the dry seed, but on the decomposition of glucosides in the presence of moisture by the enzyme *myrosin*. This decomposition results in the formation of a very pungent essential oil, which is composed almost entirely of allyl isothiocyanate, $CH_2:CH.CH_2.N:C:S$, together with traces of allyl cyanide ($CH_2:CH.CH_2CN$). Mustard also contains a fixed or saponifiable oil and a considerable amount of albuminous matter, but no starch.

Starch may be added as an adulterant, and can be detected by the microscope.

Mustard is also sometimes coloured with turmeric, and this can be extracted with alcohol and tested for in the manner described on page 151.

Salt.—Sodium chloride, which is commonly known as "salt," is one of the essential mineral constituents of a normal diet. It is found in great abundance in nature. Fine-grained or lump salt is prepared by crystallisation from brine (i.e. a saturated aqueous solution of salt) near the boiling-point.

Table salts are usually prepared from lump salt simply by grinding, and contain about 97 to 98 per cent. of sodium chloride, together with small quantities of other salts, notably calcium sulphate, magnesium sulphate, and magnesium chloride. If the latter salt is present in appreciable quantities it intensifies the salty flavour, but being hygroscopic its presence causes the salt to become lumpy and damp on exposure to moist air.

This difficulty is sometimes overcome by adding small quantities of phosphates, e.g. bone ash (calcium phosphate).

Reference should be made to the use of "iodised" salt, i.e. common salt containing a very small amount of sodium iodide. The occurrence of goitre in certain districts appears

to be associated with lack of iodide in the water supply, and in such districts the use of iodised salt is being strongly advocated.

The presence of a trace of iodide in such preparations may be shown by treating 50 gms. of the sample in a stoppered bottle with a few drops of dilute solutions of sulphuric acid and sodium nitrite. On shaking with 50 c.c. of chloroform the iodine is extracted. The amount may be determined by a method similar to that described in Vol. I., p. 233. The amounts of sodium iodide found in a number of samples examined recently in this laboratory varied from less than 1 part in 200,000 to more than 1 part in 5000.

Chapter X.

THE CALORIFIC VALUE OF FOODS.

INTRODUCTION.

It is generally agreed that for an adult, performing a limited amount of manual work, a quantity of food must be consumed during a day which, on combustion, will evolve, in the case of a man, 3000 kilogram calories, and of a woman, 2500 kilogram calories. It should be noted that these amounts of heat are often incorrectly referred to simply as 2500 or 3000 *calories*. They refer, however, to *kilogram calories*, the kilogram calorie (usually denoted Cal.) being the amount of heat required to raise the temperature of 1 kilogram of water 1° C.; 3000 Cal. are thus equal to three million calories.

The important questions of the relative proportions of fat, carbohydrate, and protein in the diet, and the presence of mineral salts and of accessory factors, are problems dealt with in other parts of the course.

In order to obtain some idea of the amount of heat represented by 3000 Cal., it is well to compare this amount of heat with that produced by the combustion of a given quantity of coal, etc.

If a sample of coal has a calorific power of 7·5 Cal. or 7500 calories per gram (i.e. 7500 Cal .per kg., which is equivalent to 13,500 B.Th.U. per lb., see Vol. I., p. 257), 3000 Cal. would be obtained by the combustion of 400 gms., i.e. about eight-ninths of a pound of this coal. A "100 calorie portion" of such a coal would be approximately 14 gms. (about half an ounce). Similarly, 333 gms. of an edible oil of calorific power 9 Cal. per gram, and 273 gms. of petrol of calorific power 11 Cal. per gram, will produce on combustion 3000 Cal. Again, if coal gas has a calorific power of 475 B.Th.U. per c. ft. (119 Cal. per c. ft.), 3000 Cal. would be obtained by the combustion of approximately 25 c. ft. of the gas, an amount which is consumed, on the average, in rather more than half an hour with a "seven radiant" gas fire.

3000 Cal. are equivalent to approximately 12,000 B.Th.U. (see Vol. I., p. 240), so that 1 *therm* (100,000 B.Th.U.) is equivalent to approximately 25,000 Cal., i.e. the heat produced by the combustion of the food eaten by a man in rather more than *eight* days.

Further, since 1 Board of Trade unit of electricity is equivalent to 864 Cal. (see Vol. I., p. 261), rather less than 4 of these units of electricity are equivalent to 3000 Cal.

The products of complete combustion of carbohydrates and fats, namely, carbon dioxide and water, are the same whether the substance is burnt with production of a flame as in a calorimeter, or oxidised slowly at a low temperature, as in the body. Not only are the final products of oxidation of these substances independent of the mode of oxidation, but the total amount of heat evolved by the combustion of the substance is independent of the process whereby the oxidation is effected. That is, a given weight of carbohydrate or fat will, on complete combustion in a calorimeter, give the same amount of heat as is obtained by the complete oxidation of the same weight of carbohydrate or fat in the body.

With proteins, the nitrogenous constituents of foods, the case is somewhat different. When a nitrogenous organic substance is burnt in oxygen, the nitrogen is either set free as nitrogen gas or, under certain conditions, a small amount of nitric acid is formed. On the other hand, when a protein is taken as food most of the nitrogen, originally contained in the substance, eventually leaves the body in the form of urea, $CO(NH_2)_2$, a substance which on combustion in oxygen would give carbon dioxide, water, and nitrogen with evolution of heat.

More heat will therefore be obtained by the combustion of a given weight of protein in a calorimeter than is obtained from that weight of protein when taken as food.

The chemical equations representing the complete combustion of the carbohydrate glucose and the fat tristearin are as follows :—

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

(Glucose)
180 gms.

$$C_{57}H_{110}O_6 + \frac{163}{2}O_2 \rightarrow 57CO_2 + 55H_2O$$

(Tristearin)
890 gms.

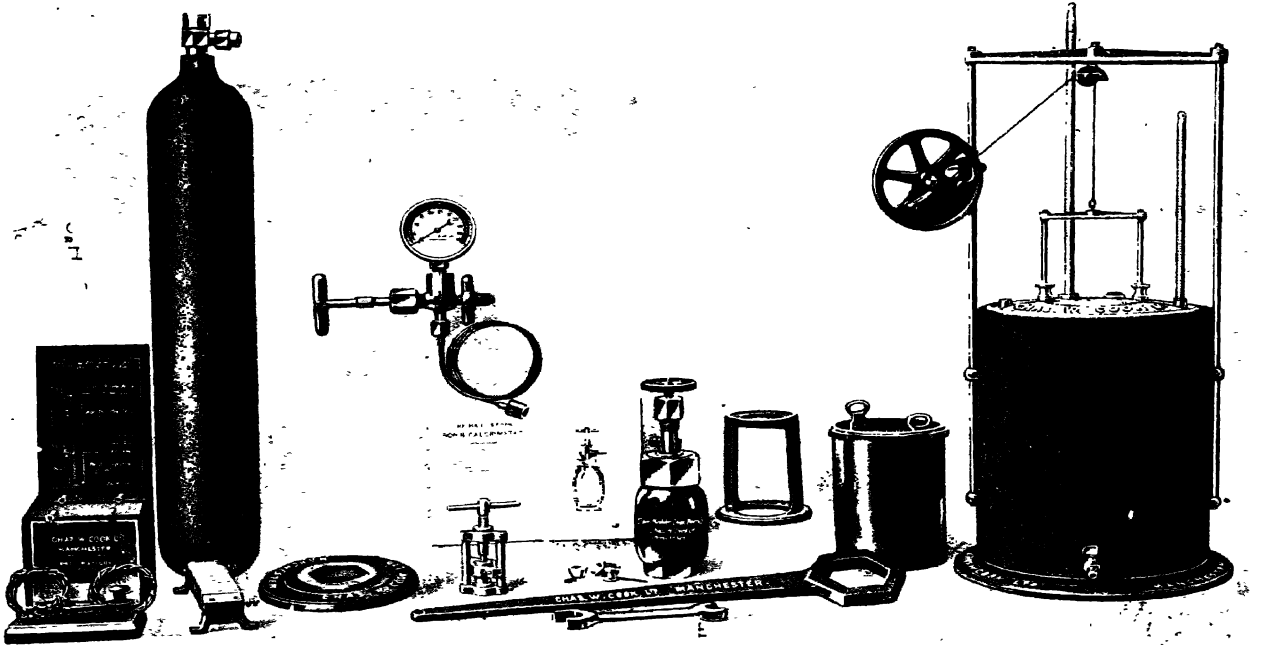


FIG 18.—Mahler-Cook Bomb Calorimeter and Accessories

[*To face p.* 259

The heat values for the corresponding thermochemical equations can be found by experiment, by burning a small amount (about 1 gm.) of the sugar or fat in a calorimeter, in such a way that the heat produced can be measured, and then calculating the amount of heat which would be generated by the combustion of 1 gm. molecule of the sugar (180 gms.) and fat (890 gms.) respectively.

It is found that on the average 1 gm. of carbohydrate gives, on combustion in a calorimeter, 4·1 Cal., 1 gm. of fat 9·45 Cal., and 1 gm. of protein 5·65 Cal.

Allowing for losses in digestion of 2, 5, and 8 per cent. in the case of carbohydrates, fats, and proteins respectively, and for the fact that combustible substances are among the end products of the disintegration of proteins in the body, it is usually stated that 1 gm. of *carbohydrate* or *protein* taken as food furnishes 4 Cal., and 1 gm. of *fat* 9 Cal. It will be seen, therefore, that two and a quarter times as much heat is obtained from a given weight of fat as is obtained from the same weight of carbohydrate or protein.

A day's food consisting of 400 gms. of carbohydrate, 100 gms. of fat, and 100 gms. of protein will thus produce on combustion in the body—

$$(400 \times 4) + (100 \times 9) + (100 \times 4) = 2900 \text{ Cal.}$$

Since the calorific power of materials used as food is obviously of such importance, the method by which these values are obtained is described in detail.

THE BOMB CALORIMETER.

It is found in connection with the determination of the calorific power of a substance (Vol. I., p. 252) that with a calorimeter constructed on the principle of the William Thomson apparatus a small amount of the substance sometimes escapes complete combustion, and consequently a low value for the calorific power is obtained.

In dealing with the calorimetry of foods, a so-called bomb calorimeter is employed, and it is only by use of this form of apparatus that trustworthy results can be obtained. One form of bomb calorimeter is shown in Figs. 18 and 19.

By means of this apparatus, which is described below, combustion of a weighed amount of material is effected in a

closed vessel in oxygen under a pressure of about 25 atmospheres, and under such conditions combustion will be complete.

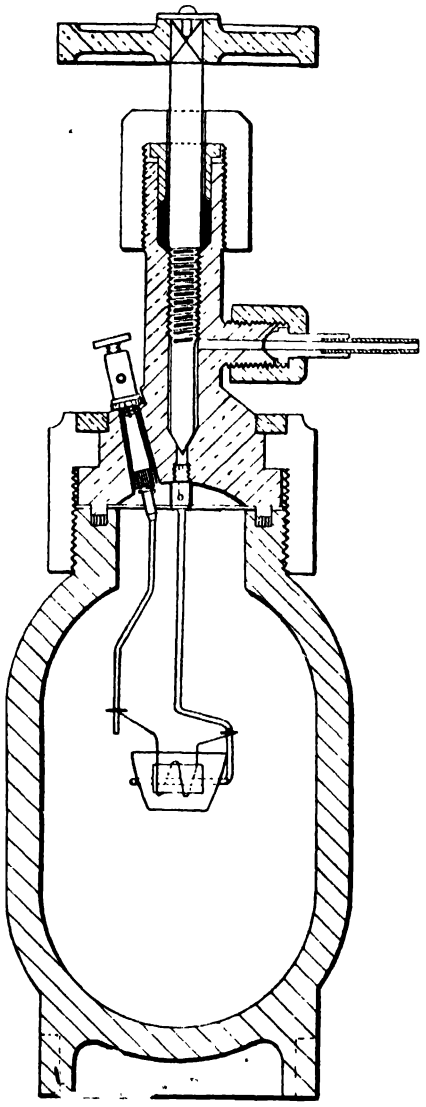


FIG. 19.—Bomb Calorimeter Section.

As in the case of other forms of calorimeter described in Vol. I., the amount of heat generated is obtained from the product of (rise of temperature) and (mass of water heated *plus* water equivalent of the calorimeter). Owing to the fact that heat is lost by radiation during the experiment, a correction known as the "radiation correction" must be made, as in the case of other calorimeters. This correction, which usually amounts to a change in temperature of about 0·02 to 0·04° C., is added to the observed rise of temperature. That is, the maximum temperature which would have been reached by the calorimeter, etc., after combustion has been effected, if heat were not lost by radiation, is from 0·02° to 0·04° C. higher than the maximum temperature actually recorded. The method by which allowance is made for this correction is dealt with later.

In determining the calorific value of a substance used as a food, it is necessary that the material should be quite dry, so that ignition is

easily effected and combustion complete. If the water content of the substance has been determined it is then possible to calculate the calorific value of the substance in the moist condition in which it is used as a food.

The Bomb.

The vessel in which the material under investigation is burnt (*the bomb*) is made of steel, nickel plated on the outside, and has a capacity of about 650 c.c. The walls are about half an inch thick, and the inner surface is covered with enamel, or preferably with gold or platinum, to protect the steel from corrosion. A section of the bomb is shown in Fig. 19. The cover of the bomb is fitted with an inlet valve for the admission of oxygen, and has two stout nickel wires attached to its lower side, which are connected to terminals on the cover. One of these wires and its terminal are insulated from the cover, otherwise short-circuiting would take place. The cover of the bomb is fastened on by means of a strong hexagon nut, and this nut can be screwed up and tightened by means of the large spanner without turning the whole cover. Whilst the cover is being fitted the bomb is held in a stand screwed to the bench, the stand fitting round the lower hexagonal part of the bomb.*

It is usual for the bomb to have been tested to withstand a pressure of about 250 atmospheres, although in an experiment with the apparatus oxygen is employed at a pressure of only 25 atmospheres. During the combustion, however, the pressure will rise above 25 atmospheres.

One of the thick wires attached to the cover is bent into a loop, to form a support for the platinum crucible in which the material under examination is to be burnt. The two thick wires are joined by a piece of very thin platinum wire (about 0·0035 inch diameter) just above the crucible, and for ignition of the material this wire is made red-hot by passing a current of electricity through it from a 4-volt accumulator, contact being made by means of a tapping key.

The Calorimeter Vessels.

The inner vessel in which the bomb is placed has a capacity of about 3 litres, and is made of copper plated with nickel.

* The spanners and stand for the hexagonal nuts should be lined with adhesive plaster to protect the nickel plating of the nuts when these are screwed up.

It rests on corks in the outer vessel, so as to provide a non-conducting air space between the two vessels. In the experiment a measured amount of water is placed in the inner vessel, so that the hexagon nut of the bomb is just covered, about 2600 c.c. being required.

The outer calorimeter vessel is a double-walled, copper vessel filled with water and covered with a thick layer of felt on the outside, to prevent heat exchange with the surrounding air.

The Stirrer and Thermometer.

It is necessary that the water in the inner calorimeter vessel should be efficiently stirred throughout the whole experiment. This is effected by means of a stirrer, which fits round the bomb and is attached by means of a cord to the large pulley, as shown in the diagram, Fig. 18.

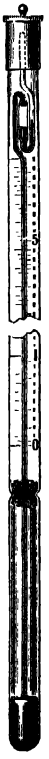


FIG. 20.—Beckmann Thermometer.

It will be noticed that a portion of this wheel is solid, and its direction of rotation should be such that the solid portion is descending while the stirrer is rising, and *vice versa*. In this way the stirrer moves up and down at a uniform rate.

By means of suitable pulley wheels driven by a motor, the stirrer is made to move up and down once in approximately a second. The length of stroke of the stirrer should be adjusted so that the upper blade does not leave the water at its highest position, otherwise splashing may take place, and heat be lost by evaporation of water from the blade.

The temperature of the water in the inner vessel is taken by means of a Beckmann thermometer (Fig. 20) reading to $\frac{1}{100}$th degree Centigrade, and set so as to allow a rise of temperature of 4 or 5 degrees. For method of setting the thermometer, see "Chemistry of Petroleum," by Tinkler and Challenger.

The Pellet Press.

It is necessary to compress a solid into the form of a pellet before ignition, otherwise some of the substance may escape combustion. In one form of press a block of iron about 2·5 × 1·5 × 1·5 ins., having a

circular hole through it, about a quarter of an inch in diameter, is placed on a small steel plate, and the hole filled with the powdered material, great care being taken that the powder taken for analysis is representative of the whole bulk of the material. A steel rod, having a diameter slightly less than that of the hole, is then forced in by means of a screw, and the powder thereby compressed. The steel plate is removed and the pellet forced through the hole.

The pellet is placed in the weighed platinum crucible, and the increase in weight gives the amount of material taken for the experiment.

Determination of the Heat of Combustion (or Calorific Value) of a Substance by means of the Bomb Calorimeter.

Outline of Method.—The platinum crucible containing the weighed pellet (about 1 gm. of material) is fixed into the ring at the end of one of the thick wires attached to the top of the bomb. A length of about 5 cm. of platinum ignition wire, made into a spiral (round a thin glass rod), is fastened from this wire, above the top of the crucible, to the other thick wire, care being taken to make good contact between the wires. A length of about 6 cm. of No. 60 sewing cotton is then tied to the thin platinum wire, and the ends of the cotton placed under the pellet. The platinum ignition wire is also bent down so as to touch the pellet; 10 c.c. of water are placed in the bomb, the cover screwed on, and the hexagon nut tightened by means of the large spanner, care being taken not to move the pellet and cotton.

The pressure gauge is attached to the oxygen cylinder (from which the ordinary reducing valve has been removed) and the bomb connected to the gauge by means of the flexible metal tubing, the union nuts being tightened by means of the small spanner. The oxygen cylinder must contain gas under a pressure greater than 25 atmospheres, or obviously the bomb cannot be filled to this pressure.

The *inlet valve of the bomb is opened, and the valve attached to the pressure gauge* (the gradual release valve) *closed.*

The oxygen cylinder valve is then opened. The pressure gauge valve (gradual release valve) is now opened *slowly* and oxygen allowed to enter the bomb, the admission of oxygen to the bomb being controlled by means of this valve. A

sudden inrush of oxygen to the bomb must be avoided, or some of the material may be thrown out of the crucible.

When the pressure gauge indicates a pressure of 25 atmospheres in the bomb, the three valves, of oxygen cylinder, pressure gauge, and bomb are closed (*in the order given*). The flexible metal tube is then disconnected.

No leakage of oxygen should be observed when the bomb is immersed in water, although a very slight leak will probably not lead to an incorrect result.

A slight leak at the insulated terminal, or at the gas inlet can often be stopped by tightening up the screws, but the greatest care should be exercised in dealing with any of the nuts when the bomb is charged with oxygen. It is occassionally necessary to replace the lead wire used as a washer for making a gas-tight joint between the bomb and its cover.

2500 c.c. (or more) of water are then placed in the inner calorimeter vessel and the bomb introduced. If the water does not cover the hexagon nut of the bomb, more water should be added from a measuring cylinder until this is the case, the amount of water added being noted.

The same quantity of water must be used in all experiments with the bomb.

The flexible leads from the battery are connected to the binding screws on the bomb, one of the leads being connected through the tapping key. Care must be taken, of course, that the circuit is not completed by making a wrong electrical connection at this point, or premature firing of the charge in the bomb may take place.

The inner calorimeter vessel is then placed in the outer vessel, previously filled with water, the lid of the calorimeter is replaced, the flexible electric leads passing through the hole in the lid. The Beckmann thermometer is now placed in position, so that its bulb is well below the surface of the water, and after an interval of about five minutes the stirring is begun.

After this stirring has proceeded for five to ten minutes, the temperature indicated by the thermometer is read every minute for a further period of five minutes. This constitutes the " First Period " referred to in connection with the *calculation* of the radiation correction. The temperature indicated by the thermometer will probably be found to remain constant during this period, or to vary only by about 0·01° C. If this is not the case, readings should be taken every minute for

further periods of five minutes, until the temperature is constant within this limit.

The ignition of the material in the calorimeter is then effected by pressing down the tapping key for about one second. The temperature is taken every minute until the maximum temperature has been reached (Second Period), and then each minute for a further period of seven minutes (Third Period)

Radiation Correction.—In spite of the precautions taken to prevent loss of heat from the apparatus by radiation, a small amount of heat will be lost in this way. Various methods have been adopted for the *calculation* of the amount of this correction from the readings of the thermometer in the *first*, *second*, and *third* periods of the experiment. This correction is dealt with fully in "Fuel: Gaseous, Liquid, and Solid," by Coste (Griffin).

For the present purpose, however, a correction which is sufficiently accurate may be obtained graphically by plotting temperatures as ordinates and time intervals as abscissæ. If the points denoting the temperatures in the third period, when the rate of cooling is approximately constant, are joined and the straight line so obtained produced backwards to cut the perpendicular through the point at which the combustion started, the point of intersection can be taken to represent the maximum temperature which would have been reached if no loss of heat by radiation had taken place.

A small correction for the heat produced by the combustion of the cotton could be made,* but if the same length is taken in the determination of the water equivalent of the calorimeter as in the determination of the heat of combustion, this amount of heat and the heat produced by passing the current through the thin platinum wire for ignition may be neglected.

Determination of the Water Equivalent of the Apparatus.—For this purpose a weighed pellet (about 1 gm.) of a pure substance of known calorific power is employed. Naphthalene (9688 calories per gram), benzoic acid (6325 calories per

* Cellulose has a calorific power of 4200 calories per gram, i.e. 4·2 calories per milligram. Six cm. of No. 60 cotton weigh approximately 2 mg., so that the correction to be made for the heat produced by the cotton is only approximately 8 calories. If the substance burnt has a calorific power of 4000 calories per gram, and 1 gram is burnt, the error in neglecting the heat produced by combustion of the cotton would be only 0·2 per cent., and less still if the calorific power of the substance is greater than 4000 calories.

gram), or cane sugar (3955 calories per gram) can be employed for this purpose. In a particular experiment, carried out as previously described, results were obtained as follows :—

Weight of platinum crucible = 2·1428 gms.
Weight of platinum crucible + benzoic acid = 3·2636 ,,
∴ Weight of benzoic acid taken = 1·1208 ,,
Initial temperature (Beckmann thermometer reading) = 2·268° C.
Final temperature corrected for radiation (see p. 265) = 4·408° ,,
∴ Rise of temperature = 2·14° ,,

Calorific power of benzoic acid = 6325 calories per gram.
∴ Heat generated = 1·1208 × 6325 = 7089 calories.
This amount of heat would raise the temperature of 3313 gms. of water 2·14° C., i.e. 3313 × 2·14 = 7089 calories.

Weight of water in calorimeter vessel and bomb = 2610 gms.

∴ 3313 − 2610 = 703 gms. of water is the water equivalent of the apparatus.

At the end of the experiment the oxygen should be allowed to escape *by opening the valve of the bomb*, after which the bomb itself may be opened and washed out with a very dilute solution of sodium hydroxide solution, followed by distilled water, and then dried. In order to keep the bomb dry a tube containing granular calcium chloride should be kept in it when not in use.

Determination of the Calorific Power of a Substance.—The experiment is carried out in exactly the same way as in the determination of the water equivalent of the apparatus described above.

If the water equivalent of the apparatus is 703 gms., then the total amount of water heated (2610 + 703 = 3313 gms.) multiplied by the rise of temperature (corrected for radiation) gives the number of calories generated by the combustion of the known weight of the substance. From this the amount of heat obtained by the combustion of 1 gm. of the substance is calculated.

If the substance contains nitrogen and sulphur, nitric and sulphuric acids will be produced. For corrections to be

applied on this account, see "Chemistry of Petroleum," by Tinkler and Challenger.

In an experiment with olive oil, results were obtained as follows :—

Weight of olive oil taken = 0·6272 gm.
Weight of water employed = 2610 gms.
Water equivalent of calorimeter = 703 ,,
Initial temperature, 2·622° C.
Final temperature corrected (as described p. 265), 4·404° ,,
Rise of temperature, 1·782° ,,
Heat evolved (2610 + 703) × 1·782 = 5903·8 calories.
∴ Heat evolved by combustion of 1 gm. of olive oil

$$\frac{5903{\cdot}8}{0{\cdot}6272} = 9413 \text{ calories,}$$

or, the heat of combustion of the given sample of olive oil is 9·41 Cal. per gram.

The thermometer readings during the progress of the experiment are given in the following table. The observed maximum was corrected as described on page 265 :—

INITIAL TEMPERATURE, 2·622°.

Intervals of One Minute after Firing and Corresponding Temperatures.

(*Second and Third Periods.*)

1	3·75°	7	4·37°
2	4·32°	8	4·365°
3	4·372°	9	4·36°
4	4·380°	10	4·355°
5	4·378°	11	4·35°
6	4·378°	12	4·345°

If the calorific power of a substance such as cooked potato is required, then, as mentioned previously, allowance must be made for the water content.

A portion of cooked potato, as eaten, was found to contain 75 per cent. of water (found by drying the material to constant weight in a steam oven), and the *dried* material was found to have a calorific value of 3·624 Cal. per gram.

Since 25 gms. of dried potato corresponds to 100 gms. of the cooked potato,

1 gm. of dried potato corresponds to $\frac{1 \times 100}{25} = 4$ gms. of the cooked potato,

or, 1 gm. of cooked potato corresponds to $\frac{1}{4}$ gm. of dried material. So that 1 gm. of *cooked* potato has a calorific value of $\frac{3{\cdot}624}{4} = 0{\cdot}906$ Cal., or, a 100 Calorie portion of this sample of cooked potato $= \frac{1 \times 100}{0{\cdot}906} = 110$ gms.

For data with regard to the calorific values of various foods, see "Chemistry of Food and Nutrition," by Sherman (Macmillan), and "Food and the Family," by V. H. Mottram (Nisbet & Co.).

BOOKS OF REFERENCE.

"Applied Chemistry," Vol. I., Tinkler and Masters (Crosby Lockwood).
"Food Inspection and Analysis," Leach (Wiley).
"Fatty Foods," Bolton and Revis (Churchill).
"Dairy Chemistry," Richmond (Griffin).
"Technology of Bread-making," Jago (Simpkin, Marshall & Co.).
"Microscopy of Vegetable Foods," Winton.
"Chemical Technology of Oils, Fats, and Waxes," Lewkowitsch (Macmillan).
"Commercial Organic Analysis," Allen (Churchill).
"Food and Drugs," Parry (Scott, Greenwood).
"Dictionary of Applied Chemistry," Thorpe (Longmans).
"Chemistry of Petroleum," by Tinkler and Challenger (Crosby Lockwood).

Other books of reference are referred to in the text.

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